



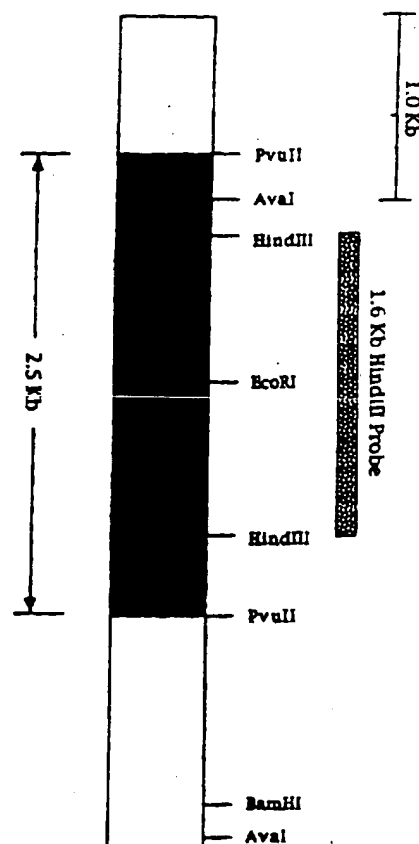
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US94/12444 <b>(22) International Filing Date:</b> 28 October 1994 (28.10.94) <b>(71) Applicant:</b> PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 700 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US). <b>(72) Inventors:</b> ALBERTSEN, Marc, C.; 1930 South 60th Street, West Des Moines, IA 50266 (US). BEACH, Larry, R.; 3939 Maquoketa Drive, Des Moines, IA 50311 (US). HOWARD, John; 2976 N.W. 132nd Court, Des Moines, IA 50311 (US). HUFFMAN, Gary, A.; 1431 41st Street, Des Moines, IA 50311 (US). <b>(74) Agents:</b> SWEENEY, Patricia, A. et al.; 700 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US).		<b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).  <b>Published</b> <i>With international search report.</i>

**(54) Title:** NUCLEOTIDE SEQUENCES MEDIATING MALE FERTILITY AND METHOD OF USING SAME

**(57) Abstract**

Nucleotide sequences mediating male fertility in plants are described, with DNA molecule and amino acid sequences set forth. Use of the nucleotide sequences to mediate fertility in plants is also described. In one such method, an inducible promoter is used to regulate expression of the DNA molecule. The control sequences are modified so that it is normally "off" and as a result the plants are male sterile. When it is desired to reproduce the male sterile plants, male fertility is restored by treating the plants with a non-phytotoxic chemical which induces expression of the critical gene.



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NUCLEOTIDE SEQUENCES MEDIATING MALE FERTILITY  
AND METHOD OF USING SAME

5  
REFERENCE TO PRIOR APPLICATION

This application is a continuation-in-part of previously filed and copending U.S. application serial  
10 number 537,183, filed June 12, 1990.

BACKGROUND OF THE INVENTION

15 The goal of plant breeding is to combine in a single variety/hybrid various desirable traits of the parental lines. For field crops, these traits may include resistance to diseases and insects, tolerance to heat and drought, reducing the time to crop maturity, greater yield, and better agronomic quality. With mechanical harvesting of  
20 many crops, uniformity of plant characteristics such as germination and stand establishment, growth rate, maturity, and fruit size, is important.

Field crops are bred through techniques that take advantage of the plant's method of pollination. A plant is  
25 self-pollinating if pollen from one flower is transferred to the same or another flower of the same plant. A plant is cross-pollinated if the pollen comes from a flower on a different plant.

In Brassica, the plant is normally self sterile and can  
30 only be cross-pollinated. In self-pollinating species, such as soybeans and cotton, the male and female plants are anatomically juxtaposed. During natural pollination, the male reproductive organs of a given flower pollinate the female reproductive organs of the same flower.

35 Maize plants (Zea mays L.) present a unique situation in that they can be bred by both self-pollination and cross-

pollination techniques. Maize has male flowers, located on the tassel, and female flowers, located on the ear, on the same plant. It can self or cross pollinate. Natural pollination occurs in maize when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears.

A reliable method of controlling male fertility in plants would offer the opportunity for improved plant breeding. This is especially true for development of maize hybrids, which relies upon some sort of male sterility system.

The development of maize hybrids requires the development of homozygous inbred lines, the crossing of these lines, and the evaluation of the crosses. Pedigree breeding and recurrent selection are two of the breeding methods used to develop inbred lines from populations. Breeding programs combine desirable traits from two or more inbred lines or various broad-based sources into breeding pools from which new inbred lines are developed by selfing and selection of desired phenotypes. A hybrid maize variety is the cross of two such inbred lines, each of which may have one or more desirable characteristics lacked by the other or which complement the other. The new inbreds are crossed with other inbred lines and the hybrids from these crosses are evaluated to determine which have commercial potential. The hybrid progeny of the first generation is designated  $F_1$ . In the development of hybrids only the  $F_1$  hybrid plants are sought. The  $F_1$  hybrid is more vigorous than its inbred parents. This hybrid vigor, or heterosis, can be manifested in many ways, including increased vegetative growth and increased yield.

Hybrid maize seed is typically produced by a male sterility system incorporating manual detasseling. Alternate strips of two inbred varieties of maize are planted in a field, and the pollen-bearing tassels are removed from one of the inbreds (female). Providing that

there is sufficient isolation from sources of foreign maize pollen, the ears of the detasseled inbred will be fertilized only with pollen from the other inbred (male), and the resulting seed is therefore hybrid and will form hybrid plants. Unfortunately, the manual detasseling process is not entirely reliable. Occasionally a female plant will be blown over by a storm and escape detasseling. The natural variation in plant development can also result in plants tasseling after manual detassling is completed. Or, a detasseler will not completely remove the tassel of the plant. In either event, the female plant will successfully shed pollen and some female plants will be self-pollinated. This will result in seed of the female inbred being harvested along with the hybrid seed which is normally produced.

Alternatively, the female inbred can be mechanically detasseled. Mechanical detasseling is approximately as reliable as manual detasseling, but is faster and less costly. However, most detasseling machines produce more damage to the plants than manual detasseling. Thus, no form of detasseling is presently entirely satisfactory, and a need continues to exist for alternatives which further reduce production costs and the eliminate self-pollination in the production of hybrid seed.

The laborious detasseling process can be avoided by using cytoplasmic male-sterile (CMS) inbreds. Plants of a CMS inbred are male sterile as a result of factors resulting from the cytoplasmic, as opposed to the nuclear, genome. Thus, this characteristic is inherited exclusively through the female parent in maize plants, since only the female provides cytoplasm to the fertilized seed. CMS plants are fertilized with pollen from another inbred that is not male-sterile. Pollen from the second inbred may or may not contribute genes that make the hybrid plants male-fertile. Usually seed from detasseled normal maize and CMS produced seed of the same hybrid must be blended to insure that

adequate pollen loads are available for fertilization when the hybrid plants are grown.

There can be other drawbacks to CMS. One is an historically observed association of a specific variant of CMS with susceptibility to certain crop diseases. This problem has led to virtual abandonment of use of that CMS variant in producing hybrid maize.

Another form of sterility, genic male sterility, is disclosed in U.S. Patents 4,654,465 and 4,727,219 to Brar et al. However, this form of genetic male sterility requires maintenance of multiple mutant genes at separate locations within the genome and requires a complex marker system to track the genes and make use of the system convenient. Patterson also described a genic system of chromosomal translocations which are effective, but complicated. U.S. Patents No. 3,861,709 and 3,710,511.

Many other attempts have been made to improve on these drawbacks. For example, Fabijanski, et al., developed several methods of causing male sterility in plants (see EPO 89/3010153.8 publication no. 329,308 and PCT application PCT/CA90/00037 published as WO 90/08828). One method includes delivering into the plant a gene encoding a cytotoxic substance associated with a male tissue specific promoter. Another involves an antisense system in which a gene critical to fertility is identified and an antisense to the gene inserted in the plant. Mariani, et al. also shows several cytotoxin encoding gene sequences, along with male tissue specific promoters and mentions an antisense system. See EP 89/401,194. Still other systems use "repressor" genes which inhibit the expression of another gene critical to male sterility. PCT/GB90/00102, published as WO 90/08829.

As noted, an essential aspect of much of the work underway with male sterility systems is the identification of genes impacting male fertility.

Such a gene can be used in a variety of systems to control male fertility. Previously, a male sterility gene has been identified in Arabidopsis thaliana and used to produce a male sterile plant. Aarts, et al., "Transposon  
5 Tagging of a Male Sterility Gene in Arabidopsis", Nature, 363:715-717 (June 24, 1993). In the present invention the inventors provide a novel DNA molecule and the amino acid sequence it encodes which is critical to male fertility in plants.

10 Further, the inventors present a unique variation to the method of controlling male sterility by using the DNA molecule to cause a plant to be male sterile after transformation, with fertility, not sterility, induced.

Thus, one object of the invention is to provide a  
15 nucleic acid sequence, the expression of which is critical to male fertility in plants.

Another object of the invention is to provide a DNA molecule encoding an amino acid sequence, the expression of which is critical to male fertility in plants.

20 A further object of the invention is to provide a method of using such DNA molecules to mediate male fertility in plants.

A still further object is to provide a method of mediating male fertility in plants by regulating expression  
25 of the DNA molecule naturally occurring in the plant.

Yet another object is to provide a method of mediating male fertility in plants by delivering the DNA molecule into a plant such that expression of the DNA molecule may be controlled.

30 Another object is to provide plants wherein male fertility of the plants is mediated by the DNA molecule.

A further object is to use plants having male fertility mediated by the DNA molecules in a plant breeding system.

Further objects of the invention will become apparent  
35 in the description and claims that follow.

## SUMMARY OF THE INVENTION

This invention relates to nucleic acid sequences, and, specifically, DNA molecules and the amino acid encoded by the DNA molecules, which are critical to male fertility. It also relates to use of such DNA molecules to mediate fertility in plants. One such method is to controllably render plants male sterile by using an inducible promoter to regulate expression of the DNA molecule such that the gene is normally "off" and the plant is thus sterile. When the promoter is induced, the plant becomes fertile.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a restriction map of the transposon Ac.

Fig. 2 is a gel of a Southern Blot analysis of PvuII digested DNA from an Ac family segregating for sterility and hybridized with an internal 1.6 kb HindIII from Ac.

Fig. 3 is a schematic representation of inverse polymerase chain reaction.

Fig. 4 is a graphic representation of the 1.4kb DNA isolate and its intervening sequences.

Fig. 5 is a Southern Blot analysis gel of PvuII digested DNA of an Ac family segregating for sterility and hybridized with the 1.4 kb DNA isolate.

Fig. 6 is a Northern Blot analysis gel hybridized with the male fertility gene MS45.

Fig. 7 shows the nucleotide and amino acid sequence of fertile revertant plant DNA after Ac transposition.

Fig. 8 is an RFLP map of chromosome 9 showing the male fertility gene MS45.



**DISCLOSURE OF THE INVENTION**

All references referred to are incorporated herein by reference.

5 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Unless mentioned otherwise, the techniques employed or contemplated herein are standard  
10 methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not limiting.

**MALE FERTILITY DNA MOLECULES**

15 Genetic male sterility results from a mutation in one of the genes responsible for a specific step in microsporogenesis, the term applied to the entire process of pollen formation. These genes can be collectively referred  
20 to as male fertility genes. There are many steps in the overall pathway where a mutation can lead to male sterility. This seems aptly supported by the frequency of genetic male sterility in maize. New alleles of male sterility mutants are uncovered in materials that range from elite inbreds to  
25 unadapted populations. To date, published genetic male sterility research has been mostly descriptive. Some efforts have been made to establish the mechanism of sterility in maize, but few have been satisfactory. This should not be surprising given the number of genes that have  
30 been identified as being responsible for male sterility. One mechanism is unlikely to apply to all mutations.

The invention is of a plant male fertile gene. cDNA's specific for pollen development and tassel development have been extensively reported. To date, none of them have led  
35 to cloning a gene that can be referred to as impacting pollen development.

The following is presented by way of illustration and is not intended to limit the scope of the invention.

### Tagging

5

Ac (Activator) is a well known transposable element first characterized in 1954 by Barbara McClintock, (McClintock, B., Cold Spring Harbor Symp. Quant. Biol. 21:197-216 (1956); McClintock, B., Carnegie Inst. Wash. Yrbook, 53:254-260 (1954); see also Federoff, U.S. Pat. No. 4,732,856 issued March 22, 1988 and Dooner, U.S. Pat. No. 5,013,658 issued May 8, 1991). Ac was used to clone this DNA molecule. A restriction map of Ac used here is depicted in Figure 1. Those skilled in the art are familiar with the restriction sites of Ac. In sum, The Ac transposon went from the P-vv locus on chromosome 1 to chromosome 9. The only currently described male sterility gene on chromosome 9 is ms2, which has never been cloned or sequenced. See Albertsen, M. and Phillips, R.L, "Developmental cytology of 13 genetic male sterile loci in maize" Canadian Jnl. of Genetics and Cytology 23:195-208 (Jan, 1981). The only cloned fertility gene is the Arabidopsis gene described. Aarts, et al., supra. Test cross progeny have confirmed the genes are not allelic.

25

### Plant Materials

Three maize lines were used, all of which are widely available to maize geneticists and regularly used by those skilled in the art and are described at Chen, et al., "Transposition of Ac from the P locus of maize into unreplicated chromosomal sites" Genetics 117:109-116 (September 1987). Such lines may be obtained, for example, from the authors of the above article, from Pioneer Hi-Bred International, Inc., or any one of many public sources such as the Maize Genetics Stock Cooperation Center, University

of Illinois, Urbana/Champagne, Department of Agronomy S-123  
Turner Hall, 1102 South Goodwin Avenue, Urbana, Illinois,  
61801.

The first line is W23P-vv. The P-vv allele is caused  
5 by the insertion of the mobile element Ac into the P locus.  
Emerson, R. "The inheritance of a recurring somatic  
variation in variegated ears of maize" Am. Nat 48:87-115  
(1914); Brink, R. and Nilan, R. "The relation between light  
variegated and medium variegated pericarp in maize" Genetics  
10 37:519-544 (1952) and Barclay, P. and Brink, R. "The  
relation between modulator and Activator in maize" Proc.  
Nat'l. Acad. Sci. USA 40:1118-1126 (1954). The P gene is a  
maize gene well characterized and fully detailed in the art.  
The P gene induces pigmentation of the pericarp in maize.  
15 Flavanone is reduced to phlobaphenes which cause  
pigmentation of the pericarp. One example of the detailed  
information on the P gene which is available to one skilled  
in the art is the discussion by Lechelt, et al., "Isolation  
and molecular analysis of the maize P locus," Mol. Gen.  
20 Genet. 219:225-234 (1989) and Chen, et al., "Molecular  
Analysis of Ac transposition and DNA replication" Genetics.  
This is an excellent marker gene because of its function in  
regulating the color of pericarp, and red striped pericarp  
results. The red stripes show the excision of Ac from P,  
25 restoring gene function and providing red pericarp.

The P-gene (P-vv) is on the same chromosome as known  
genetic male steriles previously mapped to chromosome 1. It  
has been shown that Ac transposes on the same chromosome 67%  
of the time. Van Schaik, N.V. and Brink, R.A.,  
30 "Transpositions of modulator, a component of the variegate  
pericarp allele in maize" Genetics 44:725-738 (1959).  
However, this did not occur here, as the Ac transposed to  
chromosome 9. P-vv itself greatly facilitates transposon  
tagging because it is possible to visually observe when Ac  
35 has transposed from the P-gene and is elsewhere in the  
genome.

4C063 is a white inbred line that combines well with W23P-vv to give good hybrid plants with easily scored kernels. W22r-sc:m3 is a line with the Ds element at the R-locus. The plant is genetically dominant at all the anthocyanin pathway genes (A1, A2, Bz1, Bz2, C1, C2, Pr, R). Because Ds causes R to become dysfunctional, no anthocyanin are produced in the kernel.

This was coupled with use of W22r-sc:m3 stocks, in which Ds is integrated into the R-gene. The Ds element responds to the presence of Ac, by transposing to another site on the genome. It is, in fact, a defective Ac. The Ac transposon can move in and out of a gene on its own, whereas Ds cannot move unless Ac is present somewhere on the genome. The R gene is a gene in maize studied in considerable depth. It is known to encode enzymes required for synthesis of anthocyanin pigments. An example of the detailed information known regarding the R gene is the description and sequencing information found at Dellaporta, et al., Stadler Symposium 18:263 (1988) and Ludwig, et al., "Lc, a member of the maize R gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the myc-homology region", Proc. Nat. Acad. Sci. 86:7092-7096 (Sept. 1989) and use of the gene as a visual marker, described at Bowen, et al., "R Genes as visual markers for corn transformation" Abstract edit. Gallagher, Academic Press (Oct. 1989) and Ludwig, et al., "A regulatory gene as a novel visible marker for maize transformation" Science 247: 449-450 (Jan. 26, 1990).

In the W22 r-sc:m3 stock, all kernel anthocyanin genes are dominant. The kernel color is yellow, however, because of Ds interrupting function of the R-gene. In the presence of Ac, however, the Ds element can transpose, resulting in purple-spotted kernels. Therefore, it was possible to 1) visually determine when Ac transposed away from the P-gene (red-striped or full red pericarp) and 2) determine whether

Ac was still active (purple spots in the aleurone). By selecting either all red kernels or kernels with red pericarp stripes over the embryo that also have purple spots in the aleurone, it was possible to greatly enrich for those cases where an active Ac has transposed to another location in the the genome. By selfing plants resulting from these kernels, one can screen progeny families for any mutations affecting tassel or anther development. In this case, selfed families for the segregation of male-sterile plants were created.

### Co-Segregation Analysis

Conducting co-segregation analysis for specific gene tagging and cloning strictly through a molecular approach can be tedious and time-consuming. The Ac-system, however, is well suited to co-segregation analysis at the field genetics level. Interaction between active Ac and Ds at the R-gene (r-sc:m3) can be utilized. Plants crossed with Ac were selfed and grown and those families segregating for male sterility identified. Once a family was identified that segregated for male sterility, additional seed was planted to cross with r-sc:m3 for co-segregation analysis. Each plant (fertiles and steriles) was crossed with r-sc:m3, the kernel color segregation observed on each ear and correlated with whether the plants were male fertile or male sterile.

A family was observed where the plants were mostly male sterile, with a few extruded abnormal anthers scattered about the tassel. In most cases, these abnormal anthers did not have pollen present. When every plant from this family was crossed with r-sc:m3, co-segregation of Ac with the male-sterile phenotype was observed as set forth in the table below.

TABLE 1Segregation of trhn-90-40 crossed with r:m3

5	Plant	Ear	Observed	Expected
	<u>Phenotype</u>	<u>Phenotype</u>	<u>Number</u>	<u>Number</u>
	Sterile	all kernels	8	8.25
		purple spotted		
10	Fertile	1/2 kernels	16	16.50
		purple spotted		
		1/2 kernels		
		no spots		
15	Fertile	all kernels	9	8.25
		no spot		

Male-sterile plants always produced ears with every  
 20 kernel purple spotted. Two thirds of the fertile plants had  
 ears that segregated 50% spotted kernels and 50% yellow  
 kernels. One third of the fertiles produced ears with all  
 yellow kernels. This showed Ac had transposed into a gene  
 responsible for male fertility and interrupted its function.  
 25 The gene acts as a recessive, and when homozygous, results  
 in male sterility. This segregation was verified in further  
 plantings.

Molecular Analysis

30

Southern analysis was carried out to confirm  
 association of Ac with sterility. Southern analysis is a  
 well known technique to those skilled in the art. This  
 common procedure involves isolating the plant DNA, cutting  
 35 with restriction endonucleases and fractionating the cut DNA  
 on an agarose and transferring to nitro cellulose membranes

to separate the DNA by molecular weight. It was then hybridized with the probe fragment which was radioactively labeled with P32 and washed in an SDS solution. Southern, E., "Detection of a specific sequences among DNA fragments by gel electrophoresis," J. Mol. Biol. 98:503-517 (1975).

DNA was isolated from sterile-crossed progeny and fertile-crossed progeny, keeping the purple-spotted kernel seedlings separate from the yellow kernel seedlings. DNA was isolated from the top two leaves of one month old plants using an Urea procedure as described at Dellaporta, et al., "A plant DNA minipreparation: version II" Plant Mol. Bio. Rep. 1:19-21 (1983). The isolated DNA was cut with PvuII in order to find a 2.5 kb fragment only associated with Ac as shown in the restriction map (Fig. 1). Approximately 8 ug of DNA was digested with the appropriate enzyme according to the manufacturer's instructions (Promega). DNA digests were electrophoresed through a 0.75% Sea Kem GTG agarose gel and transferred to Duralon-UV nylon membrane by capillary blotting and fixed to the membrane by baking 1 hour at 85C. The 1.6kb HindIII fragment of Ac was used as a probe in the Southern Blot analysis.

The results are shown in the gel at Figure 2. At Figure 2, the male steriles are lanes 3-10. Lane 2 is the heterozygous fertile plant and lane 1 the wild type. As this gel confirms, a 2.5 kb fragment band appeared in all sterile (purple spotted kernels) plants and did not appear in any of the fertile (yellow kernels) plants. This confirms the Ac was either closely linked to the male fertility locus or inserted into the locus, inhibiting the function of the gene and resulting in a male sterile phenotype.

### Cloning

The DNA adjacent to the known Ac sequence was cloned and used in obtaining the entire gene.

To summarize, the male fertile plant DNA and the male-sterile plant DNA were digested with restriction endonucleases Pst I, Eag I, Sal I, Sac I, and Xba I to locate a single band with the Ac element. Fragments were electrophoresed, Southern transferred, and hybridized with the Ac HindIII fragment. A 6kb PstI fragment was indentified that co-segregated with male-st element. The inverse PCR method of Baker et al was used to isolate the DNA associated with Ac. Earp, D.J. Lowe, B. and Baker B., "Amplification of genomic sequences flanking transposable elements in host and heterologous plants: a tool for transposon tagging and genomic characterization," Nucleic Acids Research 18:3271-3279 (1990).

A schematic depicting the well known inverse polymerase chain reaction procedure is shown in Figure 3. After obtaining the 6kb fragment, the ends were religated. A and B primers were identified readily since the sequence of Ac is known. Thus the 5' and 3' oligonucleotides could be identified, and, according to the inverse PCR technique, react to amplify the intervening sequences. The A and B primers were run from each side of the religated circle where the Ac had been. In this way, the DNA between the end of the Ac was amplified and a 1.3kb segment of DNA isolated. The known 4.8 kb Ac fragment plus the amplified 1.3 kb IPCR product nearly equaled the 6.0 kb Pst I fragment isolated previously.

Details of this above summarized procedure are as follows. Genomic DNA was isolated as described above. 20 ug of DNA was digested with 20 units of PstI according to the manufacturer's instructions (Promega). The digested DNA was electrophoresed as described above using a preparative comb. A gel fragment, which contained DNA with a molecular weight between 5.5 and 6.5 kilobases, was excised from the gel. The DNA was electro-eluted from the agarose by using Spectra/Por membrane #2, MWCO 12-14000 (Spectrum Medical Industries, Inc.) which contained 0.4 ml sterile water and



electrophoresing against 1X Tris-Acetate buffer pH 8.0 (TAE). The isolated DNA was extracted consecutively with Tris-equilibrated phenol pH 7.0:chloroform (1.1), chloroform, then ethanol precipitated, dried and resuspended in sterile water. Ligations were performed according to the manufacturer's instructions (Bethesda Research Laboratories) using the PstI digested genomic DNA at a final concentration of 20 ng/u. Ligations were done 18 hours at 14C.

Oligonucleotide primers were synthesized on an Applied Biosystems model 394 DNA/RNA synthesizer. Primer B5 was essentially the same as described by Earp et al., supra, except for an EcoRI site engineered at the 5' end and an extra two bases at 3' end. The sequence of both primers used in the Ac inverse PCR reaction are as follows:

A5: 5' GATAGAATTCGGTACGGGATTTTCCCATCCTACTT 3'

B5: 5' GGTAGAATTCGTTTTTCGTTTCCGTCCCGCAAGTT 3'

PCR was carried out using 25ng of circularized genomic template DNA in a reaction containing 2uM of each primer, .24mM of each dNTP, 3 units of Hot Tub polymerase (Amersham) in a 1X reaction buffer supplied by the manufacturer. Amplification was performed in a MJ Research Inc. model PTC-100-96 thermocycler under the same conditions as described by Earp et al., supra. Reaction products were electrophoresed on 1% LMP agarose gels (Bethesda Research Laboratories). The amplification product was isolated from the gel using a Magic PCR kit (Promega) and re-amplified using the above conditions.

#### cDNA Isolation

cDNA library screenings are commonly known among those skilled in the art, and are described at Mariatis T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). Libraries

were created as follows. RNA from Z. mays tassels was isolated using a guanidine thiocyanate method followed by banding in a cesium chloride gradient. Poly A+RNA was selected using oligo dT cellulose. Two cDNA libraries were constructed in the vectors pCDNAII (Invitrogen) and Uni-Zap XR (Stratagene) using 5 ug of mRNA for each according to the manufacturer's instructions.

The 1.3 inverse PCR product was probed onto the arrayed cDNA tassel library of about 1000 clones and from this a single homologous clone with an insert size of about 1.4kb obtained. It was 1550 base pairs and is graphically depicted in Figure 4. The genomic piece will, of course, vary according to the background of the plant from which it is isolated and the introns may or may not be present. This, however, shows how the Ac element appeared in this isolate.

The 1.4kb was hybridized to the PvuII segregation membrane to insure the 3.4kb co-segregating band found with the inverse PCR product was a new genomic region and not small amounts of Ac DNA contained on the ends of the fragment. The results are shown in the gel in Figure 5. As can be seen, the 1.4kb from the library hybridized in sterile plants to the same 3.4kb fragment that co-segregated with the male sterile phenotype and the purple spotted kernels plants from the fertile heterozygous.

The 1.4kb segment was then used against a second cDNA tassel library and the full length cDNA was obtained, and named MS45.

### 30 Northern Analysis

Tissue from tassels, ears and leaves of sterile and fertile plants was isolated as described previously, and a Northern Blot analysis run on the extracts. Northern analysis is also a commonly used technique by those skilled in the art and is similar to Southern analysis except that

RNA is isolated and place on an agarose gel. The RNA is then hybridized with a labelled probe. Potter, E., et al., "Thyrotropin releasing hormone exerts rapid nuclear effects to increase production of the primary prolactin mRNA transcript," Proc. Nat. Acad. Sci. USA 78:6662-6666 (1981); Lechelt, et al., supra. Total RNA was isolated from 1) leaves of plants grown approximately 2 months; 2) tassels at roughly the mid-vaculate stage; and 3) immature ears between 4.5 - 5.0 cm in length. Tissue was ground in liquid nitrogen then sequentially treated with a detergent extraction, a differential LiCl precipitation, and an ethanol precipitation. The gel was hybridized with the MS45cDNA isolated as described above. The cDNA hybridized only with DNA from fertile tassels as can be seen in Fig. 6.

### Revertants

To further confirm the gene as one critical to male fertility, revertants were identified. Since it would not be possible to distinguish normally fertile plants from revertants, plants were selected that showed sterility, but shed some pollen. These were crossed as males to unrelated lines and no male sterile plants resulted. The MS45 cDNA was recovered and analyzed to find the Ac had left a "footprint" when transposing out of the gene of six base pairs, keeping the sequence in frame. See Figure 7, showing two amino acids are added, but the frame does not shift.

### RFLP Mapping

The IPCR fragment was RFLP-mapped in a B73 X Mo17 F2 population. It mapped to chromosome 9L between probes and Burr 7.21 as described in Maize Genetics Cooperation Newsletter, 67:165 (Mar. 15, 1990) and depicted in Figure 8.

### Sequencing

Sequencing of the MS45 clone was accomplished using the dideoxy chain termination method of Sanger, et al., Proc.  
5 Nat. Acad. Sci. USA 74:5463-5464 (1977).

By referring to MS45 DNA, it is to be understood that what is meant is a DNA sequence as set forth below which produces the amino acid sequence also set forth below. One skilled in the art readily appreciates that more than are  
10 three member codon may encode the same amino acid sequence.

### METHODS OF CONTROLLING MALE FERTILITY WITH MS45 DNA

15 It is evident to one skilled in the art that the DNA described herein can be used in any one of a wide variety of methods to control male fertility in plants. The following are presented by way of illustrating several of these methods and are not intended to limit the possible uses of  
20 the DNA molecules herein described, nor the scope of the invention.

Once one has a DNA molecule that is critical to male fertility in plants, it is possible to create a sequence which is in inverse orientation to the 5' to 3' normal  
25 orientation of that DNA sequence. When this antisense molecule is delivered into the plant, it prevents normal expression of the male fertile sequence. It is believed the antisense DNA transcribes to produce an RNA which is complimentary to and capable of hybridizing to the mRNA  
30 produced by the male fertility gene and thus inhibit translation. The protein coded for by the mRNA is not produced and cannot play its role in male fertility. With the male fertility gene described herein, a construct is delivered to the plant having the MS45 DNA therein, the  
35 construct having a transcriptional promoter segment, a transcriptional termination segment and a DNA segment

producing an ribonucleotide sequence complimentary to a ribonucleotide sequence of the MS45 DNA.

This use of antisense to inhibit or control expression of a gene is known to one skilled in the art and is described in detail at Inouye, U.S. Patent 5,190,931, issued March 2, 1993. In one embodiment, the inventors there describe cutting the DNA with restriction endonucleases, to result in a religated plasmid having lost a fragment between two restriction sites and into which another DNA fragment may be inserted. The normal DNA is digested, purified and a fragment inserted in opposite orientation. They thus inhibited expression of lpp, OmpA and OmpC in bacteria and controlled the development of coliphage SP using such constructs. An antisense RNA complimentary to the 5' leader region of the OmpA RNA, but not encompassing the Shine-Dalgarno sequence was less effective than a transcript covering the ribosome binding site and initiating codon. An extensive view of antisense regulation is provided by Claude Helene and Jean-Jacques Toulme in a review, "Specific regulation of gene expression by antisense, sense and antigene nucleic acids," Biochemica et Biophysica Acta (1990) 99-125.

Another example of antisense and its use in inhibition or control of a gene include antisense constructs to genes encoding flavonoid biosynthesis in anthers to provide male sterility. Vander Meer, et al., "Antisense inhibition of flavonoid biosynthesis in petunia anthers results in male sterility," The Plant Cell, 4:253-262 (March 1992). Antisense chalcone synthesis genes with homologous sequences to other genes expressed in anthers and a CaMV355 promoter result in male sterile white pollen. As can be seen, use of antisense to control gene expression is well known. See also e.g. Bourque, June E. and Folk, William R., "Suppression of gene expression in plant cells utilizing antisense sequences transcribed by RNA polymerase II", Plant

Molecular Biology, 19:641-647 (1992); Weintrab, et al., Trends Gen. 1:22-25 (1985).

Another method of controlling gene expression is by modification of transcriptional activators. During gene  
5 expression, the double stranded DNA is transcribed to a corresponding single-stranded messenger RNA. The sense strand separates from its antisense partner and enzymes assemble an RNA molecule that compliments the sequence on the antisense strand. The mRNA migrates to ribosomes which  
10 read the encoded information to produce amino acids.

Transcription of eukaryotic genes is influenced by various elements, including, transcriptional regulatory proteins which bind to the DNA in a sequence-specific manner. These transcriptional activators may be modified so  
15 that they bind to the DNA, but cannot perform their normal activator function. Transcriptional activators have two domains, a binding domain, and an activation domain. By altering the amino acid sequence of the transcriptional activator proteins for a gene, providing a DNA sequence  
20 which codes for the same, and delivering that DNA into the plant, expression of the target gene may be blocked. See Goff, S.A. et al. "Transactivation of the Anthocyanin pathway structural genes with wild-type and altered c1 proteins" Maize Genetics Cooperation Newsletter 64:6 (March  
25 1, 1990).

A variation on this method is the isolation of genetic suppressor elements encoding dominant negative mutant proteins or inhibitory antisense RNA by random DNA fragmentation and identified by functional selection for the  
30 phenotype associated with suppression of the target. This is what Holzmayer, et al. described in their article, "Isolation of dominant negative mutants and inhibitory antisense RNA sequences by expression selection of random DNA fragments" Nucleic Acids Research, Vol. 20, No. 4, 711-  
35 717 (Dec. 3, 1991). There they randomly fragmented bacteriophage lambda DNA to protect E. coli cells from

lambda-induced lysis. Multiple genetic suppressor elements were isolated encoding either protein or antisense RNA fragments.

5 Inhibition of normal gene expression has also been observed when additional or over expression of an endogenous gene was found to suppress gene expression. This "sense inhibition", sometimes referred to as "co-suppression" has been well documented. See e.g. Brussian, et al., "An Arabidopsis mutant with reduced level of cab 140 RNA is a  
10 result of cosuppression", The Plant Cell, 5:667-677 (June, 1993); Vander Krol et al., "Flavonoid genes in Petunia: addition of a limited member of gene capus may lead to suppression of gene expression" The Plant Cell 2:291-299 (April 1990).

15 Other means of negative control regulation include repression of gene transcription. In one system factors contain DNA binding domains but lack functional activation domains, competing with activators for binding to the same sites and blocking activation. Others heterodimerize with  
20 activators reducing either their DNA-binding affinity or ability to activate transcription. Still other repressors interact with activator factors when bound to DNA and block transactivation function. A further type of down-regulators comprises inhibitory proteins that sequester the activator  
25 in a complex that is unable to bind DNA. See reviews by Jackson, M.E., J. Cell Sci. 100:1-7 (1991); Jones, N., Curr. Biol. 1:224-226 (1991); Mitchell. P.J. and Tjian, R., Science 245:371-378 (1989).

30 Direct mutation of the endogenous gene itself will also change the male fertility gene to a male sterility gene. Irradiation causes breakage and rearrangement of the chromosomes and modification of the composition of individual genes. Exposure to x-rays is a method of gene mutation well known for sometime. See e.g., Stadler, L.J.  
35 "On the genetic nature of induced mutations in plants," Reprint, Proceedings of the Sixth International Congress of

Genetics, Vol. 1, 274-294 (1932). Other techniques include exposure to chemical mutagens such as ethyl methanesulfonate, and N-methyl-N-nitro-N-nitrosoguanidine, as was accomplished by Neuffer, M.G., and Coe Jr., E.H. on pollen grains and described in their early work at "Paraffin oil technique for treating mature corn pollen with chemical mutagens" Maydica XXIII (1978) 21-28; also, see Thurling, N. & Depittayanan, "EMS induction of early flowering mutants in spring Rape (Brassica napus)" Plant Breeding 108:177-184 (1992). Other methods include treatment with sodium azide (Rao, B. "A case of genic male sterility induced by sodium azide in Pearl Millet", Biol. Zentralbl. 104:579-521 (1985); Conger, B.V. and Carabia, J.V. "Mutagenic effectiveness and efficiency of sodium azide versus ethyl methanesulfonate in maize: induction of somatic mutations at the  $yg_2$  locus by treatment of seeds differing in metabolic state and cell population" Mutation Research 46:285-296 (1977)) and exposure to gamma radiation (Filippetti, A. and DePace, C., "Improvement of seed yield in Vicia falsa L. by using experimental mutagenesis II comparisons of gamma-radiation and ethyl-methanesulfonate (EMS) in production of morphological mutants" Euphytica 35:49-59 (1986)).

Thus, it is clearly evident to one skilled in the art, that a male fertility gene, once identified, can be used in a variety of methods to mediate male fertility in plants. The foregoing illustrates but a few such methods which can be used with a novel male fertile gene. Yet one more novel method is described below created by the inventors of this application.

30

#### Constitutive Male Sterility Method

This invention differs from conventional approaches to male sterility in plant breeding and seed production in that an inducible promoter is used to regulate expression of the gene which is known to be critical to plant male fertility.



The first step in the practice of this invention is therefore the selection of a gene on which fertility is dependent. One type are the MS45 DNA molecules described, supra.

5       The selected gene is cloned, its native promoter enabled, and the modified gene is inserted into an expression sequence with an inducible promoter responsive to external control. Preferably, the promoter is one which responds to application of a specific non-phytotoxic  
10 chemical to the plant.

      Using transformation and gene substitution, the gene is inactivated in the genome of the plant and replaced by the genetically-engineered gene incorporated into the expression sequence with the inducible promoter.

15       This invention is unique in that the process results in using the inducible promoter to induce fertility, not sterility. In this invention, the selected gene's promoter sequences are removed so that the gene is not transcribed and the plant is male sterile. When it is desired to  
20 increase the male-sterile plant, male fertility is restored by inducing expression of the critical gene. In the preferred embodiment this is accomplished by treating growing male sterile plants with a specific non-phytotoxic chemical.

25       Induction of the inducible promoter by chemical treatment will be dependent on various factors associated with the chemical treatment itself and various environmental conditions at the time of treatment. If the critical gene were normally "on," to be inactivated by chemical treatment,  
30 a treatment failure would result in self-pollination and production and sale of inbred, rather than hybrid seed. Seed laws that govern the sale of hybrid seed require a high degree of seed purity such that percentages of seed that do not conform to the hybrid specification must be kept very  
35 low. Because one maize plant can produce in excess of six million pollen granules, even a limited treatment failure

could result in a high percentage of self-pollination. For these reasons, the present invention is practiced in such a manner that the gene is normally "off" and the corresponding trait is not expressed, so that under normal conditions self-pollination cannot occur. In addition, by having the critical gene normally "off," chemical treatment is not necessary in the large-scale production of hybrid seed, so that chemical usage (and associated expense) is minimized and the risk of treatment failure is present only in the carefully controlled, limited scale production of parent seed, where self-pollination is desired. Since treatment failure in such a case results in underproduction of pollen, and since pollen is normally overproduced by a wide margin, the process of this invention for production of parent seed will tolerate a treatment failure rate as high as 70% to 80% with minimal effects on yield of parent seed.

In general, in accordance with the invention described herein, the DNA molecule herein described is incorporated into the plant along with a necessary promoter which is inducible. The plant will be sterile since the DNA molecule is not expressed and when the promoter is induced, the plant will be fertile. The native gene producing the DNA molecule product is a normally fertile plant which may be inactivated by any of a variety of methods described below, such as backcrossing or homologous recombination.

#### Inducible Promoters

In the practice of this invention the promoter region is removed from a cloned gene responsible for male fertility and is replaced with a promoter that only responds to a specific external stimulus. Thus, the gene will not be transcribed except in response to the external stimulus. As long as the gene is not being transcribed, its gene product -- which is necessary for completion of pollen development -- is not produced. This causes a breakdown in one or more

of the biochemical/physiologic pathways of pollen development, which results in male sterility. The plant can only become fertile under the specific stimulus that activates the selected promoter.

5 An example of a responsive promoter system that can be used in the practice of this invention is the glutathione-S-transferase (GST) system in maize. GSTs are a family of enzymes that can detoxify a number of hydrophobic electrophilic compounds that often are used as pre-emergent  
10 herbicides (Wiegand, et al., "Messenger RNA Encoding a Glutathione-S-Transferase Responsible for Herbicide Tolerance in Maize is Induced in Response to Safener Treatment", Plant Molecular Biology 7: 235-243, 1986). It has been discovered that treating maize seed with GSTs  
15 increases the tolerance of the maize to the herbicides. Studies have shown that the GSTs are directly involved in causing this enhanced tolerance. This action is primarily mediated through a specific 1.1 kb mRNA transcription product. In short, maize has a naturally occurring  
20 quiescent gene already present that can respond to GSTs and that can be induced to produce a gene product. This gene has already been identified and cloned. Thus, in one embodiment of this invention, the promoter is removed from the GST responsive gene and attached to the male fertility  
25 gene that previously has had its native promoter removed. This engineered gene is the combination of a promoter that responds to an external chemical stimulus and a gene responsible for successful development of fertile pollen.

### 30 Gene Introduction

Several methods are known in the art for transferring cloned DNA into maize. These include electroporation-facilitated DNA uptake by maize protoplasts (Rhodes et al.,  
35 "Genetically Transformed Maize Plants from Protoplasts", Science, Vol. 240 (8 April 1988); treatment of maize

protoplasts with polyethylene glycol (Lyznik et al., "Stable Co-Transformation of Maize Protoplasts with Gus A and Neo Genes", Plant Molecular Biology 13: 151-161, 1989); and bombardment of maize cells with DNA laden microprojectiles  
5 (Klein, et al., "Genetic Transformation of Maize Cells by Particle Bombardment", Plant Physiol. (1989) 91, 440-444) and Klein, et al., "Factors Influencing Gene Delivery into Zea Mays Cells by High-Velocity Microprojectiles", Bio/Technology Vol. 6, May 1988).

10 Each of these techniques has advantages and disadvantages. In each of the techniques, DNA from a plasmid is genetically engineered such that it contains not only the gene of interest, but also selectable and screenable marker genes. A selectable marker gene is used  
15 to select only those cells that have integrated copies of the plasmid (the construction is such that the gene of interest and the selectable and screenable genes are transferred as a unit). The screenable gene provides another check for the successful culturing of only those  
20 cells carrying the genes of interest. A commonly used selectable marker gene is neomycin phosphotransferase II (NPTII). This gene conveys resistance to kanamycin, a compound that can be added directly to the growth media on which the cells grow. Plant cells are normally susceptible  
25 to kanamycin and, as a result, die. The presence of the NPTII gene overcomes the effects of the kanamycin and each cell with this gene remains viable. Another selectable marker gene which can be employed in the practice of this invention is the gene which confers resistance to the  
30 herbicide glufosinate (Basta). A screenable gene commonly used is the b-glucuronidase gene (GUS). The presence of this gene is characterized using a histochemical reaction in which a sample of putatively transformed cells is treated with a GUS assay solution. After an appropriate incubation,  
35 the cells containing the GUS gene turn blue. Another screenable gene is a transcriptional activator for

anthocyanin biosynthesis, as described in Bowen, et al., "R Genes as visual markers for corn transformation" Abstract edit. Gallagher, Academic Press (Oct. 1989); Ludwig, et al., "A regulatory gene as a novel visible marker for maize transformation" Science 247: 449-450 (Jan. 26, 1990). This gene causes the synthesis of the pigment anthocyanin. Cells transformed with a plasmid containing this gene turn red. Preferably, the plasmid will contain both selectable and screenable marker genes.

The plasmid containing one or more of these genes is introduced into either maize protoplasts or callus cells by any of the previously mentioned techniques. If the marker gene is a selectable gene, only those cells that have incorporated the DNA package survive under selection with the appropriate phytotoxic agent. Once the appropriate cells are identified and propagated, plants are regenerated. Progeny from the transformed plants must be tested to insure that the DNA package has been successfully integrated into the plant genome.

#### Inactivation of Native Gene

It will be readily appreciated by those skilled in the art that a wide variety of methods are known to disable the native gene. Homologous recombination is but one of the methods known to those skilled in the art for rendering a native gene inoperative. Thus, when the engineered gene is homologously recombined into the plant, the native gene will be rendered inoperative. A good overview of this general process is provided by Yoder, J. I., and Kmiec, Eric, in "Progress Towards Gene Targeting in Plants", Genetic Engineering, Vol. 13 (Plenum Press, New York, 1991). At page 265 of this reference, the authors note "gene targeting can be used to silence or replace the endogenous gene with an engineered allele; thus the phenotype of the altered gene, or its regulatory sequences, can be evaluated in

planta." It is pointed out that genetic recombination takes place through breakage and reunion of DNA and the rejoining mechanism pairs the complimentary DNA sequences. (See, e.g. 271, supra).

5 A further discussion of intrachromosomal homologous recombination in plants is discussed at Peterhans, A., Schlupmann, H., Basse, C. and Paszkowski, J., "Intrachromosomal Recombination in Plants", The EMBO Journal, Vol. 9, No. 11, pp. 3437-3445, 1990.

10 A variety of different means, in addition to these specific examples, would be available to one skilled in the art. A still further example includes backcrossing, using generally accepted plant breeding techniques, to in effect "delete" the native gene. Backcrossing is often used in  
15 plant breeding to transfer a specific desirable trait from one inbred or source to an inbred that lacks that trait. This can be accomplished for example by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate  
20 gene(s) for the trait in question. The progeny of this cross is then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with  
25 selection for the desired trait, the progeny will be heterozygous for loci controlling the characteristic being transferred, but will be like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give pure breeding progeny for the  
30 gene(s) being transferred. A result of any backcrossing method is that the "native" gene is replaced by the desired gene.

A unique method is discussed in the 1991 Science magazine, reporting on prior work relating to using  
35 "transgenic scissors". This article describes a method in which scientists may remove a marker gene which is attached

to a gene having a desired trait in a plant. The "scissor," according to this method, is an enzyme obtained from a bacterial virus known as "Cre" for control of recombination. Science, p. 1457, 6 December 1991. The enzyme is capable of  
5 snipping out any DNA located between a pair of 34-base pair sequences, called lox, for locus of crossing over. This is described in further detail in the patent application filed by Du Pont, and published at WO 91/09957.

#### 10 Sterility Selection And Fertility Restoration

After the gene is introduced into a plant, the appropriate plant types are selected, that is plants that are male sterile. These plants are male sterile because the  
15 isolated and cloned male fertility gene does not have its native promoter and, therefore, is not producing its gene product that is crucial to successful pollen development. Therefore, the engineered gene acts as a recessive mutant allele of that gene. In normal plant biotechnology, once  
20 the desired genotype is identified following transformation and regeneration, the plants are selfed to recover that genotype. However, in the practice of this invention, the desired genotype cannot be selfed at the first generation because it is male sterile. To obtain progeny, fertility  
25 must be induced by spraying the plants with a compound which induces transcription of the gene by activating the altered promoter. In the case of the GST promoters, the compound is preferably a GST-inducing compound such as N,N-diallyl-2-2-dichloroacetanide. The promoter attached to the male  
30 fertility gene responds to this chemical and causes the transcription of the gene to begin. Once this occurs, the normal gene product is produced from the gene and some level of male fertility is induced. Pollen from this plant is then used to effect pollination of the original selected  
35 genotype.

Once the initial isolation and propagation of the desired genotype is completed, the procedure is more straightforward. Only inbreds that are used as female parents in hybrid crosses are transformed into male sterile variants. Once they are transformed, the amount of male sterile/female fertile seed must be increased. This is accomplished by planting in an isolated area (away from other maize pollen) and spraying with a chemical to which the promoter responds. Spraying induces the promoter to start transcription of the gene attached to it. This will produce some degree of fertility. A particular advantage of this system in comparison to systems such as that disclosed in PCT publication W089/10396 of Mariani et al (based on Intl. Appl. No. PCT/EP89/00495), in which sterility is induced, is that the treatment does not have to be 100% effective, because normally much more pollen is produced by a maize plant than is actually needed for fertilization of all available silks. Therefore, even low fertility restoration will be effective in obtaining acceptable levels of seed increase. At the same time, self-pollination does not occur in hybrid seed production because the plants of this invention are normally male sterile and must be treated to become fertile. In systems in which sterility is induced, induction of sterility must be 100% effective to avoid self-pollination when hybrid seed is produced.

All the seed harvested continues to be homozygous and sterile since the fertility is only restored in a single parent generation by treatment with the fertility inducing chemical. This seed is then used in a hybrid production field where it is used as a female parent. Because the plants are male sterile, they do not have to be detasseled. All of the hybrid plants produced from such seed are male fertile because the resulting progeny inherit one modified gene from the female parent and one normal gene from the male parent. Normal pollen production occurs.



While the foregoing illustrates the preferred embodiment of the invention, it will be appreciated that various changes can be made without departing from the spirit and scope of the invention.

5

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Pioneer Hi-Bred International, Inc.

(ii) TITLE OF INVENTION: Nucleotide Sequence Mediating

10 Male Fertility and Method of Using Same

(iii) NUMBER OF SEQUENCES: 1419

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Pioneer Hi-Bred International, Inc.

(B) STREET: 700 Capital Square, 400 Locust Street

15 (C) CITY: Des Moines

(D) STATE: Iowa

(E) COUNTRY: United States

(F) ZIP: 50309

(v) COMPUTER READABLE FORM:

20 (A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Mb  
storage

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: Microsoft Windows Notepad

25 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

30 (A) APPLICATION NUMBER: 07/537,183

(B) FILING DATE: June 12, 1990

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Sweeney, Patricia A.

(B) REGISTRATION NUMBER: 32,733

35 (C) REFERENCE/DOCKET NUMBER: 125R2-PCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (515) 248-4897  
 (B) TELEFAX: (515) 248-4844

(2) INFORMATION FOR SEQ ID NO: 1:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1419 base pairs  
 (B) TYPE: nucleotide  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: MS45 cDNA clone from Zea mays

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE :

15 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Zea mays

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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20	GCGGCGAGGG CGTGATGGCA TCGTGCAGTA CCCTCACCTG TTCTTCGCGG	100
	CCCTGGCCCT GGCCCTCCTA GTCGCGGACC CGTTCGGCCT CAGTCCGCTG	150
	GCCGAGGTCG ACTACCGGCC GGTGAAGCAC GAGCTCGCGC CGTACGGGGA	200
	GGTCATGGGC AGCTGGCCCA GAGACAATGC CAGCCGGCTC AGGCGCGGGA	250
	GGCTGGAGTT CGTCGGCGAG GTGTTCTGGGC CGGAGTCCAT CGAGTTCGAT	300
25	CTCCAGGGCC GCGGGCCGTA CGCCGGCCTC GCCGACGGCC GCGTCGTGCG	350
	GTGGATGGGC GAGGAGGCCG GGTGGGAGAC GTTCGCCGTC ATGAATCCTG	400
	ACTGGTCAGA AGAAGTCTGT GCCAATGGAG TGAAGTCAAC GACGAGGAAG	450
	CAGCACGAGA AGGAGGAGTT CTGCGGCCCG CCGCTCGGCC TGAGGTTCCA	500
	CGGGGAGACC GGCGAGCTCT ACGTCGCCGA CGCGTACTAC GGTCTCATGG	550
30	TCGTTGGCCA GAGCGGCGGC GTGGCGTCTT CCGTCGCGAG GGAAGCCGAC	600
	GGGGACCCCA TCCGGTTCGC GAACGACCTC GATGTGCACA GGAATGGATC	650
	CGTATTCTTC ACTGACACGA GCATGAGATA CAGCAGAAAG GACCATCTGA	700
	ACATCCTGTT AGAAGGAGAA GGCACCGGGA GGCTGCTCAG GTACGATCCA	750
	GAAACAAGTG CTGTCCATGT CGTGCTCAAG GGAAGTGTGT TCCCAAACGG	800
35	CGTGCAGATC TCAGAAGACC ATCAGTTTCT TCTCTTCTCC GAGACAACAA	850
	ACTGCAGGAT AATGAGGTAC TGGCTGGAAG GCCCAAGAGC GAGCGAGGTA	900

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GAGGTGTTTCG CGAACCTGCC GGGCTTCCCC GACAACGTGC GCTCCAACGG 950
CAGGGGGCCAG TTCTGGGTGG CGATCGACTG CTGCCGGACG CCAGCGCAGG 1000
AGGTGTTTCGC CAAGAGGCCG TGGCTCCGGA CCCTGTACTT CAAGTTCCCG 1050
CTGTGCTCA AGGTGCTCAC TTGGAAGGCC GCCAGGAGGA TGCACACGGT 1100
5 GCTCGCGCTC CTCGACGGCG AAGGGCGCGT CGTGGAGGTG CTCGAGGACC 1150
GGGGCCACGA GGTGATGAAG CTGGTGAGCG AGGTGCGGGA GGTGGGCAGC 1200
AAGCTGTGGA TCGGAACCGT GGC GCACAAC CACATCGCCA CCATCCCCTA 1250
CCCTTTAGAG GACTAACCAT GATCTATGCT GTTTC AATGC CTCCTAATCT 1300
GTGTACGTCT ATAAATGTCT AATGCAGTCA CTGGTTGTAA TCTTGTTTGT 1350
10 GTTTGGCAAA TTGGCATAAT AATGGACAGA TTCAATGGGC AAAAAAAAAA 1400
AAAAAAAAAA AACTCGAG 1419

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 473 base pairs

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid

20 (A) DESCRIPTION:

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE :

(vi) ORIGINAL SOURCE:

25 (A) ORGANISM: Zea mays

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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30 Gln Trp Arg Arg Gly Arg Asp Gly Ile Val Gln Tyr Pro His Leu
          20              25              30
Phe Phe Ala Ala Leu Ala Leu Ala Leu Leu Val Ala Asp Pro Phe
          35              40              45
Gly Leu Ser Pro Leu Ala Glu Val Asp Tyr Arg Pro Val Lys His
35          50              55              60
Glu Leu Ala Pro Tyr Gly Glu Val Met Gly Ser Trp Pro Arg Asp
          65              70              75

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	Asn	Ala	Ser	Arg	Leu	Arg	Arg	Gly	Arg	Leu	Glu	Phe	Val	Gly	Glu	
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	Val	Phe	Gly	Pro	Glu	Ser	Ile	Glu	Phe	Asp	Leu	Gln	Gly	Arg	Gly	
					95					100					105	
5	Pro	Tyr	Ala	Gly	Leu	Ala	Asp	Gly	Arg	Val	Val	Arg	Trp	Met	Gly	
					110					115					120	
	Glu	Glu	Ala	Gly	Trp	Glu	Thr	Phe	Ala	Val	Met	Asn	Pro	Asp	Trp	
					125					130					135	
	Ser	Glu	Glu	Val	Cys	Ala	Asn	Gly	Val	Asn	Ser	Thr	Thr	Arg	Lys	
10					140					145					150	
	Gln	His	Glu	Lys	Glu	Glu	Phe	Cys	Gly	Arg	Pro	Leu	Gly	Leu	Arg	
					155					160					165	
	Phe	His	Gly	Glu	Thr	Gly	Glu	Leu	Tyr	Val	Ala	Asp	Ala	Tyr	Tyr	
					170					175					180	
15	Gly	Leu	Met	Val	Val	Gly	Gln	Ser	Gly	Gly	Val	Ala	Ser	Ser	Val	
					185					190					195	
	Ala	Arg	Glu	Ala	Asp	Gly	Asp	Pro	Ile	Arg	Phe	Ala	Asn	Asp	Leu	
					200					205					210	
	Asp	Val	His	Arg	Asn	Gly	Ser	Val	Phe	Phe	Thr	Asp	Thr	Ser	Met	
20					215					220					225	
	Arg	Tyr	Ser	Arg	Lys	Asp	His	Leu	Asn	Ile	Leu	Leu	Glu	Gly	Glu	
					230					235					240	
	Gly	Thr	Gly	Arg	Leu	Leu	Arg	Tyr	Asp	Pro	Glu	Thr	Ser	Ala	Val	
					245					250					255	
25	His	Val	Val	Leu	Lys	Gly	Leu	Val	Phe	Pro	Asn	Gly	Val	Gln	Ile	
					260					265					270	
	Ser	Glu	Asp	His	Gln	Phe	Leu	Leu	Phe	Ser	Glu	Thr	Thr	Asn	Cys	
					275					280					285	
	Arg	Ile	Met	Arg	Tyr	Trp	Leu	Glu	Gly	Pro	Arg	Ala	Ser	Glu	Val	
30					290					295					300	
	Glu	Val	Phe	Ala	Asn	Leu	Pro	Gly	Phe	Pro	Asp	Asn	Val	Arg	Ser	
					305					310					315	
	Asn	Gly	Arg	Gly	Gln	Phe	Trp	Val	Ala	Ile	Asp	Cys	Cys	Arg	Thr	
					320					325					330	
35	Pro	Ala	Gln	Glu	Val	Phe	Ala	Lys	Arg	Pro	Trp	Leu	Arg	Thr	Leu	
					335					340					345	
	Tyr	Phe	Lys	Phe	Pro	Leu	Ser	Leu	Lys	Val	Leu	Thr	Trp	Lys	Ala	
					350					355					360	
	Ala	Arg	Arg	Met	His	Thr	Val	Leu	Ala	Leu	Leu	Asp	Gly	Glu	Gly	

	365	370	375
	Arg Val Val Glu Val Leu Glu Asp Arg Gly His Glu Val Met Lys		
	380	385	390
	Leu Val Ser Glu Val Arg Glu Val Gly Ser Lys Leu Trp Ile Gly		
5	395	400	405
	Thr Val Ala His Asn His Ile Ala Thr Ile Pro Tyr Pro Leu Glu		
	410	415	420
	Asp Xaa Pro Xaa Ser Met Leu Phe Gln Cys Leu Leu Ile Cys Val		
	425	430	435
10	Arg Leu Xaa Met Ser Asn Ala Val Thr Gly Cys Asn Leu Val Cys		
	440	445	450
	Val Trp Gln Ile Gly Ile Ile Met Asp Arg Phe Asn Gly Gln Lys		
	455	460	465
	Lys Lys Lys Lys Lys Lys Leu Glu		
15	470	473	

We claim:

Claim 1

A nucleotide sequence encoding the amino acid sequence  
5 of:

	Glu	Phe	Gly	Thr	Arg	Ser	Thr	Ser	Met	Glu	Glu	Lys	Arg	Lys	Leu	
						5					10				15	
	Gln	Trp	Arg	Arg	Gly	Arg	Asp	Gly	Ile	Val	Gln	Tyr	Pro	His	Leu	
						20					25				30	
10	Phe	Phe	Ala	Ala	Leu	Ala	Leu	Ala	Leu	Leu	Val	Ala	Asp	Pro	Phe	
						35					40				45	
	Gly	Leu	Ser	Pro	Leu	Ala	Glu	Val	Asp	Tyr	Arg	Pro	Val	Lys	His	
						50					55				60	
15	Glu	Leu	Ala	Pro	Tyr	Gly	Glu	Val	Met	Gly	Ser	Trp	Pro	Arg	Asp	
						65					70				75	
	Asn	Ala	Ser	Arg	Leu	Arg	Arg	Gly	Arg	Leu	Glu	Phe	Val	Gly	Glu	
						80					85				90	
	Val	Phe	Gly	Pro	Glu	Ser	Ile	Glu	Phe	Asp	Leu	Gln	Gly	Arg	Gly	
20						95					100				105	
	Pro	Tyr	Ala	Gly	Leu	Ala	Asp	Gly	Arg	Val	Val	Arg	Trp	Met	Gly	
						110					115				120	
	Glu	Glu	Ala	Gly	Trp	Glu	Thr	Phe	Ala	Val	Met	Asn	Pro	Asp	Trp	
						125					130				135	
25	Ser	Glu	Glu	Val	Cys	Ala	Asn	Gly	Val	Asn	Ser	Thr	Thr	Arg	Lys	
						140					145				150	
	Gln	His	Glu	Lys	Glu	Glu	Phe	Cys	Gly	Arg	Pro	Leu	Gly	Leu	Arg	
						155					160				165	
	Phe	His	Gly	Glu	Thr	Gly	Glu	Leu	Tyr	Val	Ala	Asp	Ala	Tyr	Tyr	
30						170					175				180	
	Gly	Leu	Met	Val	Val	Gly	Gln	Ser	Gly	Gly	Val	Ala	Ser	Ser	Val	
						185					190				195	
	Ala	Arg	Glu	Ala	Asp	Gly	Asp	Pro	Ile	Arg	Phe	Ala	Asn	Asp	Leu	
						200					205				210	
35	Asp	Val	His	Arg	Asn	Gly	Ser	Val	Phe	Phe	Thr	Asp	Thr	Ser	Met	
						215					220				225	

	Arg	Tyr	Ser	Arg	Lys	Asp	His	Leu	Asn	Ile	Leu	Leu	Glu	Gly	Glu	
					230					235					240	
	Gly	Thr	Gly	Arg	Leu	Leu	Arg	Tyr	Asp	Pro	Glu	Thr	Ser	Ala	Val	
					245					250					255	
5	His	Val	Val	Leu	Lys	Gly	Leu	Val	Phe	Pro	Asn	Gly	Val	Gln	Ile	
					260					265					270	
	Ser	Glu	Asp	His	Gln	Phe	Leu	Leu	Phe	Ser	Glu	Thr	Thr	Asn	Cys	
					275					280					285	
	Arg	Ile	Met	Arg	Tyr	Trp	Leu	Glu	Gly	Pro	Arg	Ala	Ser	Glu	Val	
10					290					295					300	
	Glu	Val	Phe	Ala	Asn	Leu	Pro	Gly	Phe	Pro	Asp	Asn	Val	Arg	Ser	
					305					310					315	
	Asn	Gly	Arg	Gly	Gln	Phe	Trp	Val	Ala	Ile	Asp	Cys	Cys	Arg	Thr	
					320					325					330	
15	Pro	Ala	Gln	Glu	Val	Phe	Ala	Lys	Arg	Pro	Trp	Leu	Arg	Thr	Leu	
					335					340					345	
	Tyr	Phe	Lys	Phe	Pro	Leu	Ser	Leu	Lys	Val	Leu	Thr	Trp	Lys	Ala	
					350					355					360	
	Ala	Arg	Arg	Met	His	Thr	Val	Leu	Ala	Leu	Leu	Asp	Gly	Glu	Gly	
20					365					370					375	
	Arg	Val	Val	Glu	Val	Leu	Glu	Asp	Arg	Gly	His	Glu	Val	Met	Lys	
					380					385					390	
	Leu	Val	Ser	Glu	Val	Arg	Glu	Val	Gly	Ser	Lys	Leu	Trp	Ile	Gly	
					395					400					405	
25	Thr	Val	Ala	His	Asn	His	Ile	Ala	Thr	Ile	Pro	Tyr	Pro	Leu	Glu	
					410					415					420	
	Asp	Xaa	Pro	Xaa	Ser	Met	Leu	Phe	Gln	Cys	Leu	Leu	Ile	Cys	Val	
					425					430					435	
	Arg	Leu	Xaa	Met	Ser	Asn	Ala	Val	Thr	Gly	Cys	Asn	Leu	Val	Cys	
30					440					445					450	
	Val	Trp	Gln	Ile	Gly	Ile	Ile	Met	Asp	Arg	Phe	Asn	Gly	Gln	Lys	
					455					460					465	
	Lys	Lys	Lys	Lys	Lys	Lys	Leu	Glu								
					470					473						

35

## Claim 2

An RNA molecule according to claim 1.

5

## Claim 3

A DNA molecule that mediates fertility in plants and encodes the amino acid sequence:

10

	Glu	Phe	Gly	Thr	Arg	Ser	Thr	Ser	Met	Glu	Glu	Lys	Arg	Lys	Leu	
						5				10					15	
	Gln	Trp	Arg	Arg	Gly	Arg	Asp	Gly	Ile	Val	Gln	Tyr	Pro	His	Leu	
						20				25					30	
15	Phe	Phe	Ala	Ala	Leu	Ala	Leu	Ala	Leu	Leu	Val	Ala	Asp	Pro	Phe	
						35				40					45	
	Gly	Leu	Ser	Pro	Leu	Ala	Glu	Val	Asp	Tyr	Arg	Pro	Val	Lys	His	
						50				55					60	
	Glu	Leu	Ala	Pro	Tyr	Gly	Glu	Val	Met	Gly	Ser	Trp	Pro	Arg	Asp	
20						65				70					75	
	Asn	Ala	Ser	Arg	Leu	Arg	Arg	Gly	Arg	Leu	Glu	Phe	Val	Gly	Glu	
						80				85					90	
	Val	Phe	Gly	Pro	Glu	Ser	Ile	Glu	Phe	Asp	Leu	Gln	Gly	Arg	Gly	
						95				100					105	
25	Pro	Tyr	Ala	Gly	Leu	Ala	Asp	Gly	Arg	Val	Val	Arg	Trp	Met	Gly	
						110				115					120	
	Glu	Glu	Ala	Gly	Trp	Glu	Thr	Phe	Ala	Val	Met	Asn	Pro	Asp	Trp	
						125				130					135	
	Ser	Glu	Glu	Val	Cys	Ala	Asn	Gly	Val	Asn	Ser	Thr	Thr	Arg	Lys	
30						140				145					150	
	Gln	His	Glu	Lys	Glu	Glu	Phe	Cys	Gly	Arg	Pro	Leu	Gly	Leu	Arg	
						155				160					165	
	Phe	His	Gly	Glu	Thr	Gly	Glu	Leu	Tyr	Val	Ala	Asp	Ala	Tyr	Tyr	
						170				175					180	
35	Gly	Leu	Met	Val	Val	Gly	Gln	Ser	Gly	Gly	Val	Ala	Ser	Ser	Val	
						185				190					195	



	Ala Arg Glu Ala Asp Gly Asp Pro Ile Arg Phe Ala Asn Asp Leu		
	200	205	210
	Asp Val His Arg Asn Gly Ser Val Phe Phe Thr Asp Thr Ser Met		
	215	220	225
5	Arg Tyr Ser Arg Lys Asp His Leu Asn Ile Leu Leu Glu Gly Glu		
	230	235	240
	Gly Thr Gly Arg Leu Leu Arg Tyr Asp Pro Glu Thr Ser Ala Val		
	245	250	255
	His Val Val Leu Lys Gly Leu Val Phe Pro Asn Gly Val Gln Ile		
10	260	265	270
	Ser Glu Asp His Gln Phe Leu Leu Phe Ser Glu Thr Thr Asn Cys		
	275	280	285
	Arg Ile Met Arg Tyr Trp Leu Glu Gly Pro Arg Ala Ser Glu Val		
	290	295	300
15	Glu Val Phe Ala Asn Leu Pro Gly Phe Pro Asp Asn Val Arg Ser		
	305	310	315
	Asn Gly Arg Gly Gln Phe Trp Val Ala Ile Asp Cys Cys Arg Thr		
	320	325	330
	Pro Ala Gln Glu Val Phe Ala Lys Arg Pro Trp Leu Arg Thr Leu		
20	335	340	345
	Tyr Phe Lys Phe Pro Leu Ser Leu Lys Val Leu Thr Trp Lys Ala		
	350	355	360
	Ala Arg Arg Met His Thr Val Leu Ala Leu Leu Asp Gly Glu Gly		
	365	370	375
25	Arg Val Val Glu Val Leu Glu Asp Arg Gly His Glu Val Met Lys		
	380	385	390
	Leu Val Ser Glu Val Arg Glu Val Gly Ser Lys Leu Trp Ile Gly		
	395	400	405
	Thr Val Ala His Asn His Ile Ala Thr Ile Pro Tyr Pro Leu Glu		
30	410	415	420
	Asp Xaa Pro Xaa Ser Met Leu Phe Gln Cys Leu Leu Ile Cys Val		
	425	430	435
	Arg Leu Xaa Met Ser Asn Ala Val Thr Gly Cys Asn Leu Val Cys		
	440	445	450
35	Val Trp Gln Ile Gly Ile Ile Met Asp Arg Phe Asn Gly Gln Lys		
	455	460	465

Lys Lys Lys Lys Lys Lys Leu Glu

470

473

## Claim 4

5 A DNA molecule that mediates fertility in plants comprising:

	GAATTCGGCA	CGAGGTCCAC	CAGCATGGAG	GAGAAGAGGA	AGCTGCAGTG	50
	GCGGCGAGGG	CGTGATGGCA	TCGTGCAGTA	CCCTCACCTG	TTCTTCGCGG	100
10	CCCTGGCCCT	GGCCCTCCTA	GTCGCGGACC	CGTTTCGGCCT	CAGTCCGCTG	150
	GCCGAGGTCTG	ACTACCGGCC	GGTGAAGCAC	GAGCTCGCGC	CGTACGGGGA	200
	GGTCATGGGC	AGCTGGCCCA	GAGACAATGC	CAGCCGGCTC	AGGCGCGGGA	250
	GGCTGGAGTT	CGTCGGCGAG	GTGTTTCGGGC	CGGAGTCCAT	CGAGTTCGAT	300
	CTCCAGGGCC	GCGGGCCGTA	CGCCGGCCTC	GCCGACGGCC	GCGTCGTGCG	350
15	GTGGATGGGC	GAGGAGGCCG	GGTGGGAGAC	GTTCGCCGTC	ATGAATCCTG	400
	ACTGGTCAGA	AGAAGTCTGT	GCCAATGGAG	TGAACTCAAC	GACGAGGAAG	450
	CAGCACGAGA	AGGAGGAGTT	CTGCGGCCGG	CCGCTCGGCC	TGAGGTTCCA	500
	CGGGGAGACC	GGCGAGCTCT	ACGTCGCCGA	CGCGTACTAC	GGTCTCATGG	550
	TCGTTGGCCA	GAGCGGCGGC	GTGGCGTCCT	CCGTCGCGAG	GGAAGCCGAC	600
20	GGGGACCCCA	TCCGGTTCGC	GAACGACCTC	GATGTGCACA	GGAATGGATC	650
	CGTATTCTTC	ACTGACACGA	GCATGAGATA	CAGCAGAAAG	GACCATCTGA	700
	ACATCCTGTT	AGAAGGAGAA	GGCACCAGGA	GGCTGCTCAG	GTACGATCCA	750
	GAAACAAGTG	CTGTCCATGT	CGTGCTCAAG	GGACTGGTGT	TCCCAAACGG	800
	CGTGCAGATC	TCAGAAGACC	ATCAGTTTCT	TCTCTTCTCC	GAGACAACAA	850
25	ACTGCAGGAT	AATGAGGTAC	TGGCTGGAAG	GCCCAAGAGC	GAGCGAGGTA	900
	GAGGTGTTTCG	CGAACCTGCC	GGGCTTCCCC	GACAACGTGC	GCTCCAACGG	950
	CAGGGGCCAG	TTCTGGGTGG	CGATCGACTG	CTGCCGGACG	CCAGCGCAGG	1000
	AGGTGTTTCGC	CAAGAGGCCG	TGGCTCCGGA	CCCTGTACTT	CAAGTTCCCG	1050
	CTGTCGCTCA	AGGTGCTCAC	TTGGAAGGCC	GCCAGGAGGA	TGCACACGGT	1100
30	GCTCGCGCTC	CTCGACGGCG	AAGGGCGCGT	CGTGGAGGTG	CTCGAGGACC	1150
	GGGGCCACGA	GGTGATGAAG	CTGGTGAGCG	AGGTGCGGGA	GGTGGGCAGC	1200
	AAGCTGTGGA	TCGGAACCGT	GGCGCACAAAC	CACATCGCCA	CCATCCCCTA	1250
	CCCTTTAGAG	GACTAACCAT	GATCTATGCT	GTTTCAATGC	CTCCTAATCT	1300
	GTGTACGTCT	ATAAATGTCT	AATGCAGTCA	CTGGTTGTAA	TCTTGTTTGT	1350
35	GTTTGGCAAA	TTGGCATAAT	AATGGACAGA	TTCAATGGGC	AAAAAAAAAA	1400
	AAAAAAAAAA	AAACTCGAG				1419

## Claim 5

A plasmid vector containing the nucleotide sequence of claim 1.

5

## Claim 6

A plant transformed by the nucleotide sequence of claim 1.

10

## Claim 7

A plant cell transformed by the nucleotide sequence of claim 1.

## Claim 8

15 The plant of claim 6 wherein the plant is maize.

## Claim 9

20 The plant cell of claim 7 wherein the plant cell is a maize plant cell.

## Claim 10

A method of mediating fertility of a plant comprising mediating a nucleotide sequence in the plant encoding the amino acid sequence:

Glu	Phe	Gly	Thr	Arg	Ser	Thr	Ser	Met	Glu	Glu	Lys	Arg	Lys	Leu
					5				10					15
Gln	Trp	Arg	Arg	Gly	Arg	Asp	Gly	Ile	Val	Gln	Tyr	Pro	His	Leu
					20				25					30
Phe	Phe	Ala	Ala	Leu	Ala	Leu	Ala	Leu	Leu	Val	Ala	Asp	Pro	Phe
					35				40					45
Gly	Leu	Ser	Pro	Leu	Ala	Glu	Val	Asp	Tyr	Arg	Pro	Val	Lys	His
					50				55					60
Glu	Leu	Ala	Pro	Tyr	Gly	Glu	Val	Met	Gly	Ser	Trp	Pro	Arg	Asp
					65				70					75

	Asn	Ala	Ser	Arg	Leu	Arg	Arg	Gly	Arg	Leu	Glu	Phe	Val	Gly	Glu	
					80					85					90	
	Val	Phe	Gly	Pro	Glu	Ser	Ile	Glu	Phe	Asp	Leu	Gln	Gly	Arg	Gly	
					95					100					105	
5	Pro	Tyr	Ala	Gly	Leu	Ala	Asp	Gly	Arg	Val	Val	Arg	Trp	Met	Gly	
					110					115					120	
	Glu	Glu	Ala	Gly	Trp	Glu	Thr	Phe	Ala	Val	Met	Asn	Pro	Asp	Trp	
					125					130					135	
	Ser	Glu	Glu	Val	Cys	Ala	Asn	Gly	Val	Asn	Ser	Thr	Thr	Arg	Lys	
10					140					145					150	
	Gln	His	Glu	Lys	Glu	Glu	Phe	Cys	Gly	Arg	Pro	Leu	Gly	Leu	Arg	
					155					160					165	
	Phe	His	Gly	Glu	Thr	Gly	Glu	Leu	Tyr	Val	Ala	Asp	Ala	Tyr	Tyr	
					170					175					180	
15	Gly	Leu	Met	Val	Val	Gly	Gln	Ser	Gly	Gly	Val	Ala	Ser	Ser	Val	
					185					190					195	
	Ala	Arg	Glu	Ala	Asp	Gly	Asp	Pro	Ile	Arg	Phe	Ala	Asn	Asp	Leu	
					200					205					210	
	Asp	Val	His	Arg	Asn	Gly	Ser	Val	Phe	Phe	Thr	Asp	Thr	Ser	Met	
20					215					220					225	
	Arg	Tyr	Ser	Arg	Lys	Asp	His	Leu	Asn	Ile	Leu	Leu	Glu	Gly	Glu	
					230					235					240	
	Gly	Thr	Gly	Arg	Leu	Leu	Arg	Tyr	Asp	Pro	Glu	Thr	Ser	Ala	Val	
					245					250					255	
25	His	Val	Val	Leu	Lys	Gly	Leu	Val	Phe	Pro	Asn	Gly	Val	Gln	Ile	
					260					265					270	
	Ser	Glu	Asp	His	Gln	Phe	Leu	Leu	Phe	Ser	Glu	Thr	Thr	Asn	Cys	
					275					280					285	
	Arg	Ile	Met	Arg	Tyr	Trp	Leu	Glu	Gly	Pro	Arg	Ala	Ser	Glu	Val	
30					290					295					300	
	Glu	Val	Phe	Ala	Asn	Leu	Pro	Gly	Phe	Pro	Asp	Asn	Val	Arg	Ser	
					305					310					315	
	Asn	Gly	Arg	Gly	Gln	Phe	Trp	Val	Ala	Ile	Asp	Cys	Cys	Arg	Thr	
					320					325					330	
35	Pro	Ala	Gln	Glu	Val	Phe	Ala	Lys	Arg	Pro	Trp	Leu	Arg	Thr	Leu	
					335					340					345	

Tyr Phe Lys Phe Pro Leu Ser Leu Lys Val Leu Thr Trp Lys Ala  
 350 355 360  
 Ala Arg Arg Met His Thr Val Leu Ala Leu Leu Asp Gly Glu Gly  
 365 370 375  
 5 Arg Val Val Glu Val Leu Glu Asp Arg Gly His Glu Val Met Lys  
 380 385 390  
 Leu Val Ser Glu Val Arg Glu Val Gly Ser Lys Leu Trp Ile Gly  
 395 400 405  
 Thr Val Ala His Asn His Ile Ala Thr Ile Pro Tyr Pro Leu Glu  
 10 410 415 420  
 Asp Xaa Pro Xaa Ser Met Leu Phe Gln Cys Leu Leu Ile Cys Val  
 425 430 435  
 Arg Leu Xaa Met Ser Asn Ala Val Thr Gly Cys Asn Leu Val Cys  
 440 445 450  
 15 Val Trp Gln Ile Gly Ile Ile Met Asp Arg Phe Asn Gly Gln Lys  
 455 460 465  
 Lys Lys Lys Lys Lys Lys Leu Glu  
 470 473

20

## Claim 11

The method of claim 10 wherein the nucleotide sequence mediated is an RNA molecule.

## Claim 12

25

The method of claim 10 wherein the nucleotide sequence mediated is a DNA molecule.

## Claim 13

30 A method of mediating fertility of a plant comprising mediating a DNA molecule in the plant of the sequence:

GAATTCGGCA CGAGGTCCAC CAGCATGGAG GAGAAGAGGA AGCTGCAGTG 50  
 GCGGCGAGGG CGTGATGGCA TCGTGCAGTA CCCTCACCTG TTCTTCGCGG 100  
 CCCTGGCCCT GGCCCTCCTA GTCGCGGACC CGTTCGGCCT CAGTCCGCTG 150  
 35 GCCGAGGTCG ACTACCGGCC GGTGAAGCAC GAGCTCGCGC CGTACGGGGA 200  
 GGTCATGGGC AGCTGGCCCA GAGACAATGC CAGCCGGCTC AGGCGCGGGA 250

	GGCTGGAGTT	CGTCGGCGAG	GTGTTCGGGC	CGGAGTCCAT	CGAGTTCGAT	300
	CTCCAGGGCC	GCGGGCCGTA	CGCCGGCCTC	GCCGACGGCC	GCGTCGTGCG	350
	GTGGATGGGC	GAGGAGGCCG	GGTGGGAGAC	GTTCGCCGTC	ATGAATCCTG	400
	ACTGGTCAGA	AGAAGTCTGT	GCCAATGGAG	TGAACTCAAC	GACGAGGAAG	450
5	CAGCACGAGA	AGGAGGAGTT	CTGCGGCCGG	CCGCTCGGCC	TGAGGTTCCA	500
	CGGGGAGACC	GGCGAGCTCT	ACGTCGCCGA	CGCGTACTAC	GGTCTCATGG	550
	TCGTTGGCCA	GAGCGGCGGC	GTGGCGTCTT	CCGTGCGGAG	GGAAGCCGAC	600
	GGGGACCCCA	TCCGGTTTCG	GAACGACCTC	GATGTGCACA	GGAATGGATC	650
	CGTATTCTTC	ACTGACACGA	GCATGAGATA	CAGCAGAAAG	GACCATCTGA	700
10	ACATCCTGTT	AGAAGGAGAA	GGCACCGGGA	GGCTGCTCAG	GTACGATCCA	750
	GAAACAAGTG	CTGTCCATGT	CGTGCTCAAG	GGACTGGTGT	TCCCAAACGG	800
	CGTGCAGATC	TCAGAAGACC	ATCAGTTTCT	TCTCTTCTCC	GAGACAACAA	850
	ACTGCAGGAT	AATGAGGTAC	TGGCTGGAAG	GCCCAAGAGC	GAGCGAGGTA	900
	GAGGTGTTTCG	CGAACCTGCC	GGGCTTCCCC	GACAACGTGC	GCTCCAACGG	950
15	CAGGGGCCAG	TTCTGGGTGG	CGATCGACTG	CTGCCGGACG	CCAGCGCAGG	1000
	AGGTGTTTCGC	CAAGAGGCCG	TGGCTCCGGA	CCCTGTACTT	CAAGTTCCCG	1050
	CTGTCGCTCA	AGGTGCTCAC	TTGGAAGGCC	GCCAGGAGGA	TGCACACGGT	1100
	GCTCGCGCTC	CTCGACGGCG	AAGGGCGCGT	CGTGGAGGTG	CTCGAGGACC	1150
	GGGGCCACGA	GGTGATGAAG	CTGGTGAGCG	AGGTGCGGGA	GGTGGGCAGC	1200
20	AAGCTGTGGA	TCGGAACCGT	GGCGCACAAAC	CACATCGCCA	CCATCCCCTA	1250
	CCCTTTAGAG	GACTAACCAT	GATCTATGCT	GTTTCAATGC	CTCCTAATCT	1300
	GTGTACGTCT	ATAAATGTCT	AATGCAGTCA	CTGGTTGTAA	TCTTGTTTGT	1350
	GTTTGGCAAA	TTGGCATAAT	AATGGACAGA	TTCAATGGGC	AAAAAAAAAA	1400
	AAAAAAAAAA	AAACTCGAG				1419

25

Claim 14

A method for mediating male fertility in a plant comprising causing repression of expression of a nucleotide sequence encoding the amino acid sequence:

Glu Phe Gly Thr Arg Ser Thr Ser Met Glu Glu Lys Arg Lys Leu  
 5 10 15  
 Gln Trp Arg Arg Gly Arg Asp Gly Ile Val Gln Tyr Pro His Leu  
 20 25 30  
 Phe Phe Ala Ala Leu Ala Leu Ala Leu Val Ala Asp Pro Phe

	35	40	45
	Gly Leu Ser Pro Leu Ala Glu Val Asp Tyr Arg Pro Val Lys His		
	50	55	60
	Glu Leu Ala Pro Tyr Gly Glu Val Met Gly Ser Trp Pro Arg Asp		
5	65	70	75
	Asn Ala Ser Arg Leu Arg Arg Gly Arg Leu Glu Phe Val Gly Glu		
	80	85	90
	Val Phe Gly Pro Glu Ser Ile Glu Phe Asp Leu Gln Gly Arg Gly		
	95	100	105
10	Pro Tyr Ala Gly Leu Ala Asp Gly Arg Val Val Arg Trp Met Gly		
	110	115	120
	Glu Glu Ala Gly Trp Glu Thr Phe Ala Val Met Asn Pro Asp Trp		
	125	130	135
	Ser Glu Glu Val Cys Ala Asn Gly Val Asn Ser Thr Thr Arg Lys		
15	140	145	150
	Gln His Glu Lys Glu Glu Phe Cys Gly Arg Pro Leu Gly Leu Arg		
	155	160	165
	Phe His Gly Glu Thr Gly Glu Leu Tyr Val Ala Asp Ala Tyr Tyr		
	170	175	180
20	Gly Leu Met Val Val Gly Gln Ser Gly Gly Val Ala Ser Ser Val		
	185	190	195
	Ala Arg Glu Ala Asp Gly Asp Pro Ile Arg Phe Ala Asn Asp Leu		
	200	205	210
	Asp Val His Arg Asn Gly Ser Val Phe Phe Thr Asp Thr Ser Met		
25	215	220	225
	Arg Tyr Ser Arg Lys Asp His Leu Asn Ile Leu Leu Glu Gly Glu		
	230	235	240
	Gly Thr Gly Arg Leu Leu Arg Tyr Asp Pro Glu Thr Ser Ala Val		
	245	250	255
30	His Val Val Leu Lys Gly Leu Val Phe Pro Asn Gly Val Gln Ile		
	260	265	270
	Ser Glu Asp His Gln Phe Leu Leu Phe Ser Glu Thr Thr Asn Cys		
	275	280	285
	Arg Ile Met Arg Tyr Trp Leu Glu Gly Pro Arg Ala Ser Glu Val		
35	290	295	300
	Glu Val Phe Ala Asn Leu Pro Gly Phe Pro Asp Asn Val Arg Ser		

		305		310		315
	Asn Gly Arg Gly Gln Phe Trp Val Ala Ile Asp Cys Cys Arg Thr					
		320		325		330
	Pro Ala Gln Glu Val Phe Ala Lys Arg Pro Trp Leu Arg Thr Leu					
5		335		340		345
	Tyr Phe Lys Phe Pro Leu Ser Leu Lys Val Leu Thr Trp Lys Ala					
		350		355		360
	Ala Arg Arg Met His Thr Val Leu Ala Leu Leu Asp Gly Glu Gly					
		365		370		375
10	Arg Val Val Glu Val Leu Glu Asp Arg Gly His Glu Val Met Lys					
		380		385		390
	Leu Val Ser Glu Val Arg Glu Val Gly Ser Lys Leu Trp Ile Gly					
		395		400		405
	Thr Val Ala His Asn His Ile Ala Thr Ile Pro Tyr Pro Leu Glu					
15		410		415		420
	Asp Xaa Pro Xaa Ser Met Leu Phe Gln Cys Leu Leu Ile Cys Val					
		425		430		435
	Arg Leu Xaa Met Ser Asn Ala Val Thr Gly Cys Asn Leu Val Cys					
		440		445		450
20	Val Trp Gln Ile Gly Ile Ile Met Asp Arg Phe Asn Gly Gln Lys					
		455		460		465
	Lys Lys Lys Lys Lys Lys Leu Glu					
		470		473		

25

## Claim 15

A method for mediating male fertility in a plant comprising causing repression of an expression of a DNA molecule of the sequence:

30	GAATTCGGCA CGAGGTCCAC CAGCATGGAG GAGAAGAGGA AGCTGCAGTG	50
	GCGGCGAGGG CGTGATGGCA TCGTGCAGTA CCCTCACCTG TTCTTCGCGG	100
	CCCTGGCCCT GGCCCTCCTA GTCGCGGACC CGTTCGGCCT CAGTCCGCTG	150
	GCCGAGGTCG ACTACCGGCC GGTGAAGCAC GAGCTCGCGC CGTACGGGGA	200
	GGTCATGGGC AGCTGGCCCA GAGACAATGC CAGCCGGCTC AGGCGCGGGA	250
35	GGCTGGAGTT CGTCGGCGAG GTGTTCCGGGC CGGAGTCCAT CGAGTTCGAT	300
	CTCCAGGGCC GCGGGCCGTA CGCCGGCCTC GCCGACGGCC GCGTCGTGCG	350



GTGGATGGGC GAGGAGGCCG GGTGGGAGAC GTTCGCCGTC ATGAATCCTG 400  
 ACTGGTCAGA AGAAGTCTGT GCCAATGGAG TGAAGTCAAC GACGAGGAAG 450  
 CAGCACGAGA AGGAGGAGTT CTGCGGCCGG CCGCTCGGCC TGAGGTTCCA 500  
 CGGGGAGACC GGCGAGCTCT ACGTCGCCGA CGCGTACTAC GGTCTCATGG 550  
 5 TCGTTGGCCA GAGCGGCGGC GTGGCGTCCT CCGTCGCGAG GGAAGCCGAC 600  
 GGGGACCCCA TCCGGTTTCG GAACGACCTC GATGTGCACA GGAATGGATC 650  
 CGTATTCTTC ACTGACACGA GCATGAGATA CAGCAGAAAG GACCATCTGA 700  
 ACATCCTGTT AGAAGGAGAA GGCACCGGGA GGCTGCTCAG GTACGATCCA 750  
 GAAACAAGTG CTGTCCATGT CGTGCTCAAG GGAAGTGGTGT TCCCAAACGG 800  
 10 CGTGCAGATC TCAGAAGACC ATCAGTTTCT TCTCTTCTCC GAGACAACAA 850  
 ACTGCAGGAT AATGAGGTAC TGGCTGGAAG GCCCAAGAGC GAGCGAGGTA 900  
 GAGGTGTTTCG CGAACCTGCC GGGCTTCCCC GACAACGTGC GCTCCAACGG 950  
 CAGGGGCCAG TTCTGGGTGG CGATCGACTG CTGCCGGACG CCAGCGCAGG 1000  
 AGGTGTTTCG CAAGAGGCCG TGGCTCCGGA CCCTGTACTT CAAGTTCCCG 1050  
 15 CTGTCGCTCA AGGTGCTCAC TTGGAAGGCC GCCAGGAGGA TGCACACGGT 1100  
 GCTCGCGCTC CTCGACGGCG AAGGGCGCGT CGTGGAGGTG CTCGAGGACC 1150  
 GGGGCCACGA GGTGATGAAG CTGGTGAGCG AGGTGCGGGA GGTGGGCAGC 1200  
 AAGCTGTGGA TCGGAACCGT GGCACACAAC CACATCGCCA CCATCCCCTA 1250  
 CCCTTTAGAG GACTAACCAT GATCTATGCT GTTTCAATGC CTCCTAATCT 1300  
 20 GTGTACGTCT ATAAATGTCT AATGCAGTCA CTGGTTGTAA TCTTGTTTGT 1350  
 GTTTGGCAAA TTGGCATAAT AATGGACAGA TTCAATGGGC AAAAAAAAAA 1400  
 AAAAAAAAAA AACTCGAG 1419

## Claim 16

25 The method of claim 14 wherein expression of the nucleotide sequence is repressed by mutation of the nucleotide sequence.

## Claim 17

30 The method of claim 14 further comprising delivering into the plant a second nucleotide sequence which represses expression of the nucleotide sequence.

## Claim 18

The method of claim 15 wherein expression of the DNA molecule is repressed by mutation of the DNA molecule.

5

## Claim 19

The method of claim 15 further comprising delivering into the plant a second nucleotide sequence molecule oriented in the antisense direction relative to the DNA molecule thereby repressing expression of the DNA molecule.

10

## Claim 20

The method of claim 15 further comprising delivering into the plant a second DNA molecule which represses expression of the DNA molecule.

15

## Claim 21

A male fertility mediated plant produced according to the method of claim 10.

20

## Claim 22

A male fertility mediated plant produced according to the method of claim 13.

25

## Claim 23

A method of providing heritable externally controllable male sterility in a plant comprising:

30

linking the DNA molecule of claim 3 in an expression sequence with an inducible promoter responsive to external control;

delivering the expression sequence into the genome of the plant; and

5

The method of claim 23 wherein the amino acid sequence of the DNA molecule is encoded by the DNA molecule of claim 4.

Claim 25

planting seed of the plant to provide growing male  
sterile plants;

inducing conversion of the growing plants to male  
fertile form under conditions which induce the  
promoter to express the second DNA molecule; and

25 open-pollinating the growing plants in isolation to  
produce seed; and harvesting the seed.

The method of claim 25 wherein the amino acid sequence  
30 of the DNA molecule is encoded by the DNA molecule of claim  
4.

A controllably male sterile plant produced according to  
35 the method of claim 23.

## Claim 28

A method of producing hybrid seed, comprising the steps of:

5     planting in cross-pollinating juxtaposition, a first  
      seed from a selected male fertile parent line and a  
      second seed selected from a female parent line  
      having male sterility resulting from the  
      replacement of a first native DNA molecule which  
10     encodes for the amino acid sequence set forth in  
      claim 3 with a second DNA molecule of claim 3  
      linked in an expression sequence with an inducible  
      promoter responsive to external control;

15     growing the seed to mature plants under conditions  
      which do not induce expression of the second DNA  
      molecule;

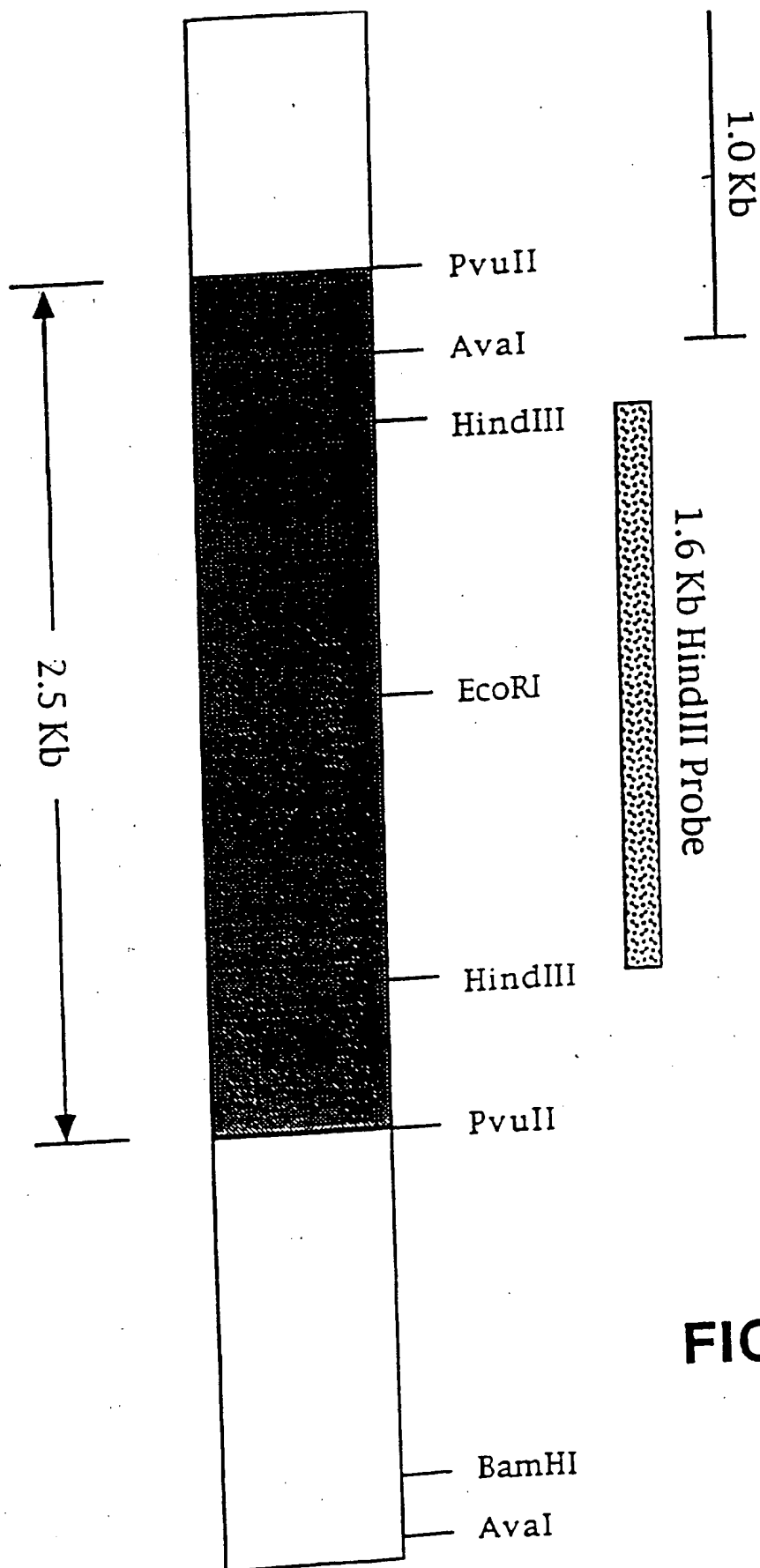
      cross-pollinating the male sterile female plant with  
20     pollen from the male fertile plant; and

      harvesting seed from the male sterile female plant.

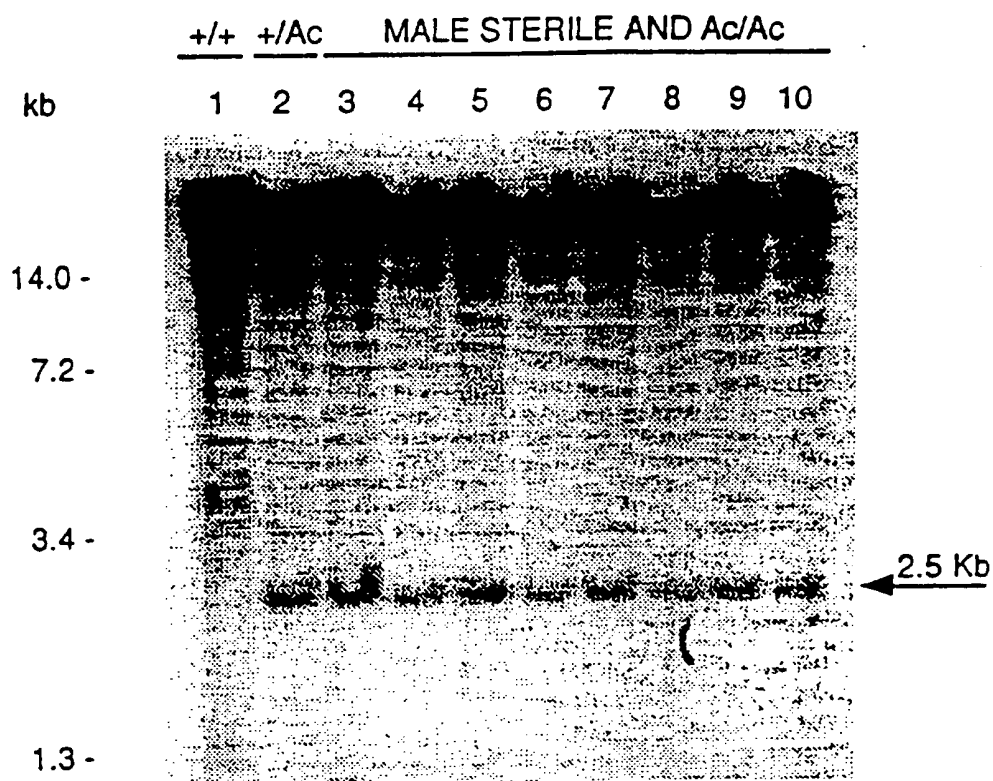
## Claim 29

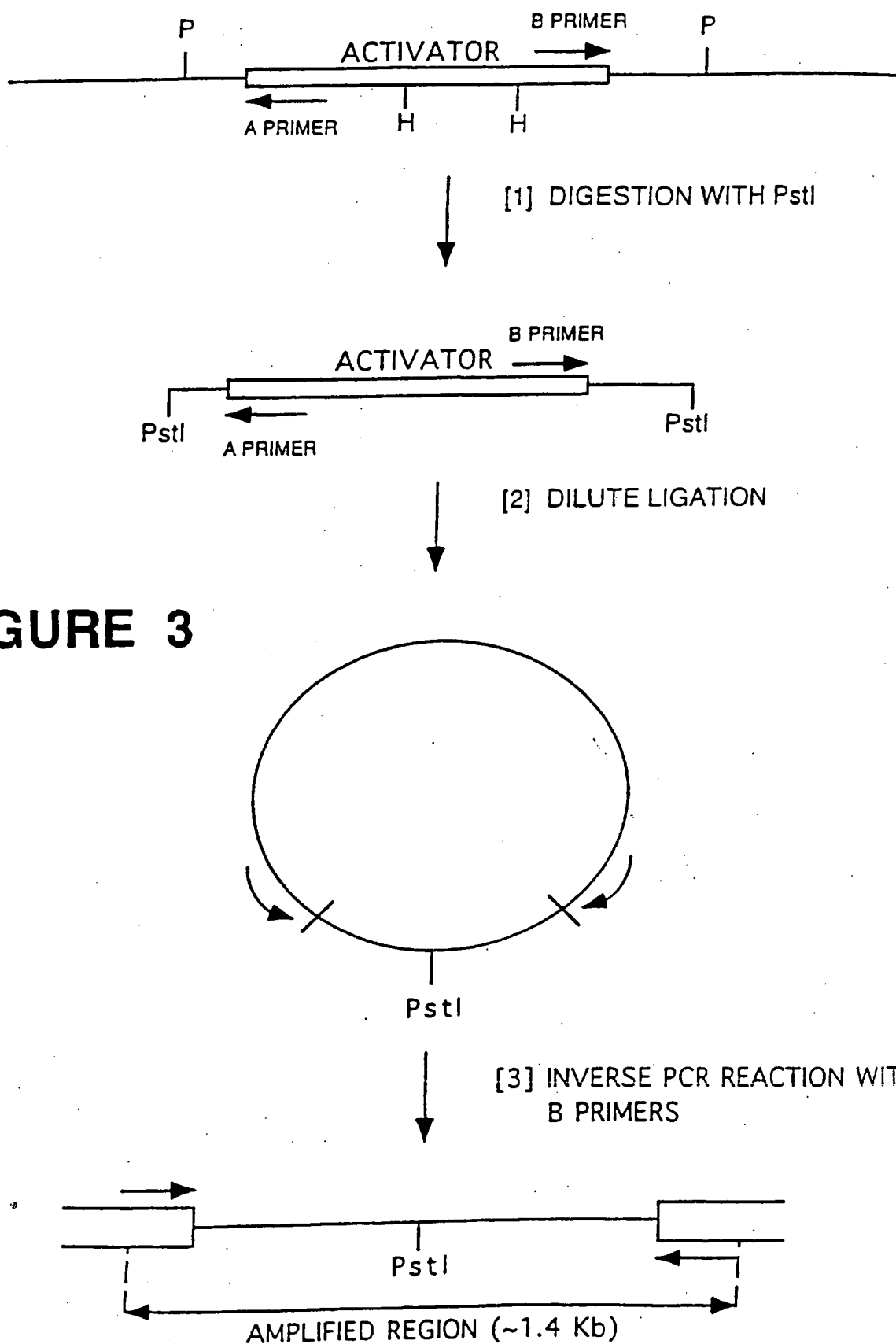
25     An expression cassette containing the nucleotide  
      sequence of claim 1 operably linked to plant regulatory  
      sequences which cause the expression of the nucleotide  
      sequences in plant cells.

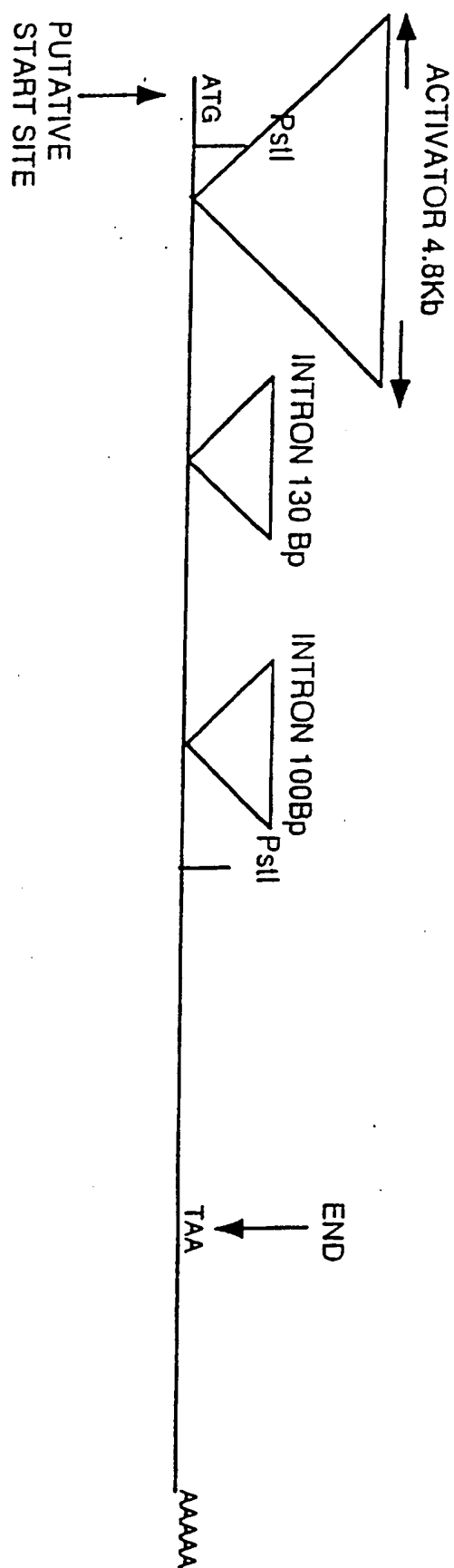
30



**FIGURE 1**

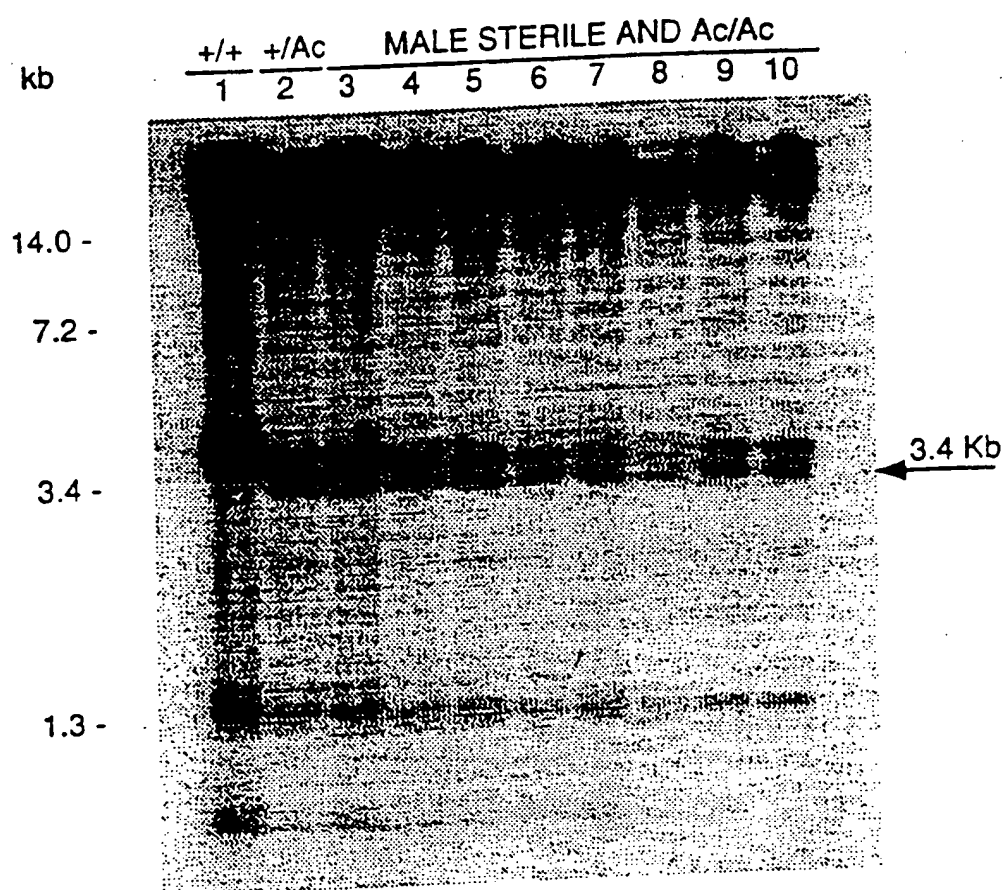
**FIGURE 2**

**FIGURE 3**

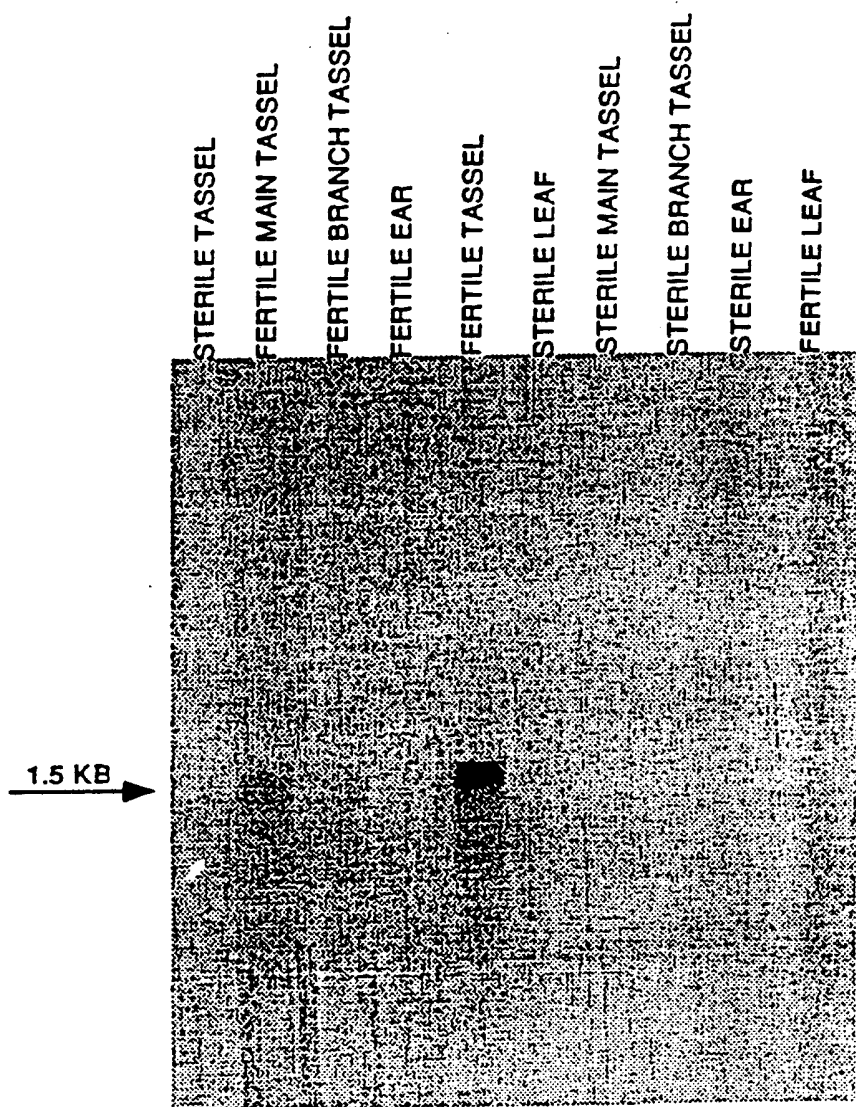


**FIGURE 4**



**FIGURE 5**

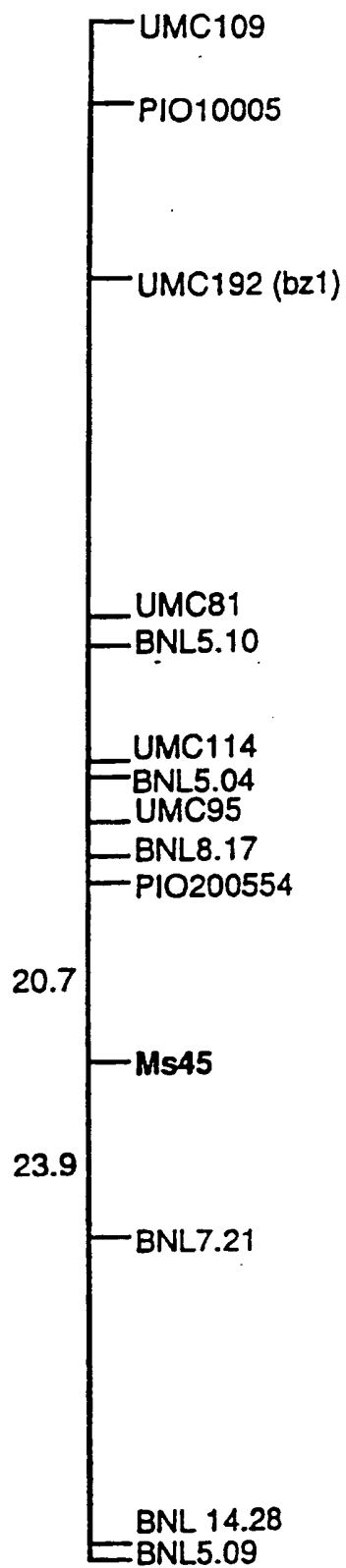
# FIGURE 6



MS45

**A L A L A L L V A V A D P F G L**  
 GCCCTGGCCCTGGCCCTCCTAGTCGCGGTGCGACCCGTTTCGGCCTC  
 |||||  
 GCCCTGGCCCTGGCCCTCCTA GTCGCGACCCGTTTCGGCCTC  
**A L A L A L L V A D P F G L**

## FIGURE 7



**FIGURE 8**

## INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 94/12444

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/83 C07K14/415 A01H1/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT CELL, vol. 6, July 1994 pages 920-925, CHASAN, R. 'MEETING REPORT: A MEETING OF MINDS ON MAIZE' see page 922, column 1	1-4
O,X	& MAIZE GENETICS CONFERENCE, HELD MARCH 24-27, 1994, CHICAGO, USA., ---	1-4
A	AM. J. BOT. 80. (6 SUPPL.).1993.16, ALBERTSEN, M.C., ET AL. 'TAGGING CLONING AND CHARACTERIZING A MALE FERTILITY GENE IN MAIZE' see abstract 44 & FOURTH JOINT MEETING OF THE BOTANICAL SOCIETY OF AMERICA AND THE CANADIAN BOTANICAL ASSOCIATION, AMES, IOWA, USA, AUGUST 1-5, 1993., --- -/--	1-4



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

30 June 1995

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, vol. 363, 24 June 1993 pages 715-717, AARTS, M.G.M., ET AL. 'TRANSPOSON TAGGING OF A MALE STERILITY GENE IN ARABIDOPSIS ' see the whole document ---	1-4
A	EP-A-0 465 024 (PIONEER HI BRED INT) 8 January 1992 see the whole document ---	10-29
A	WO-A-93 18171 (PIONEER HI BRED INT ;UNIV WASHINGTON (US)) 16 September 1993 see page 13, line 10 - page 15, line 35 ---	10-29
T	WO-A-94 25593 (CT VOOR PLANTENVEREDELINGS EN ;STIEKEMA WILLEM JOHANNES (NL); PERE) 10 November 1994 see the whole document -----	1-29

# INTERNATIONAL SEARCH REPORT

Int: ☐ International Application No

PCT/US 94/12444

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0465024	08-01-92	AU-B- 643791	25-11-93
		AU-A- 7735891	19-12-91
		CA-A- 2042447	13-12-91
		JP-A- 6046697	22-02-94
-----			
WO-A-9318171	16-09-93	EP-A- 0631630	04-01-95
		HU-A- 67838	29-05-95
-----			
WO-A-9425593	10-11-94	NONE	
-----			





# INTERNATIONAL SEARCH REPORT

Int: nal Application No

PCT/US 94/12444

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0465024	08-01-92	AU-B- 643791	25-11-93
		AU-A- 7735891	19-12-91
		CA-A- 2042447	13-12-91
		JP-A- 6046697	22-02-94
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WO-A-9318171	16-09-93	EP-A- 0631630	04-01-95
		HU-A- 67838	29-05-95
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WO-A-9425593	10-11-94	NONE	
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